Structure-based design, synthesis and preliminary evaluation of selective inhibitors of dihydrofolate reductase from *Mycobacterium tuberculosis*

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Abstract

Tuberculosis is an increasing threat, owing to the spread of AIDS and to the development of resistance of the causative organism, *Mycobacterium tuberculosis*, to the currently available drugs. Dihydrofolate reductase (DHFR) is an important enzyme of the folate cycle; inhibition of DHFR inhibits growth and causes cell death. The crystal structure of *M. tuberculosis* DHFR revealed a glycerol tightly bound close to the binding site for the substrate dihydrofolate; this glycerol-binding motif is absent from the human enzyme. A series of pyrimidine-2,4-diamines was designed with a two-carbon tether between a glycerol-mimicking triol and the 6-position of the heterocycle; these compounds also carried aryl substituents at the 5-position. These, their diastereoisomers, analogues lacking two hydroxy groups and analogues lacking the two-carbon spacing linker were synthesised by acylation of the anions derived from phenylacetonitriles with ethyl (4*S*,5*R*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate, ethyl (4*S*,5*S*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate, tetrahydrooxepin-2-one and 2,3-*O*-isopropylidene-D-erythronolactone, respectively, to give the corresponding α-acylphenylacetonitriles. Formation of the methyl enol ethers, condensation with guanidine and deprotection gave the pyrimidine-2,4-diamines. Preliminary assay of the abilities of these compounds to inhibit the growth of TB5 *Saccharomyces cerevisiae* carrying the DHFR genes from *M. tuberculosis*, human and yeast indicated that 5-phenyl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine selectively inhibited *M. tuberculosis* DHFR and had little effect on the human or yeast enzymes.

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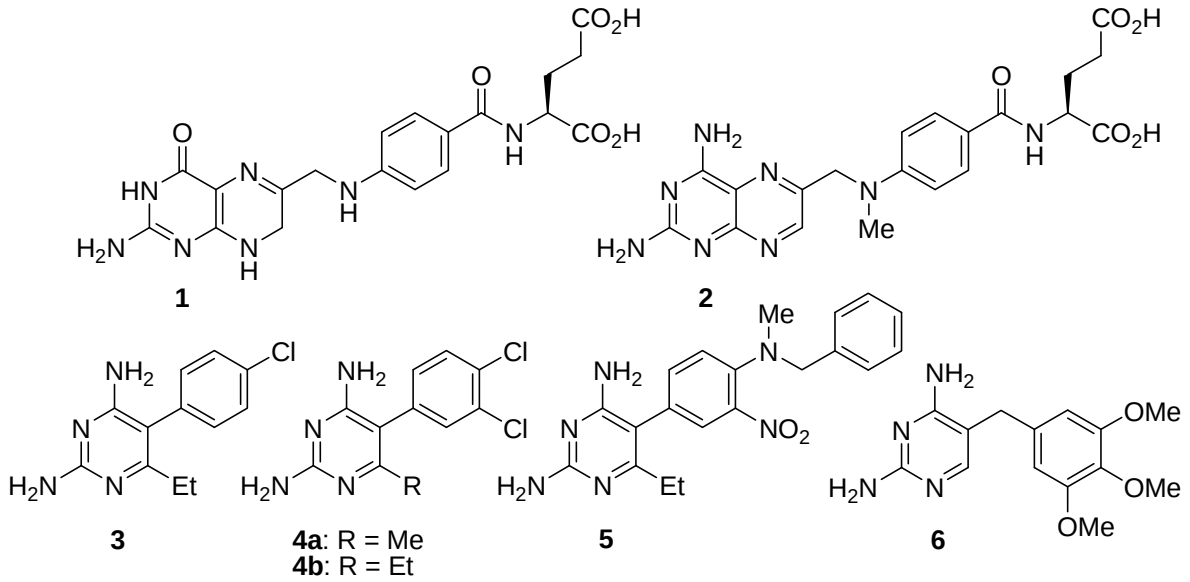


Figure 1. Structures of the DHFR substrate dihydrofolate **1** and the inhibitors methotrexate **2**, pyrimethamine **3**, DDMP / metoprine **4a**, etoprine **4b**, methylbenzoprim **5** and trimethoprim **6**.

1. Introduction

Tuberculosis (TB) is responsible for the highest number of deaths of all infectious diseases.[1] Rates of TB continue to rise, leading to an estimated eight million new cases every year and an annual death toll of two million.[2] Several factors have contributed to this increase, such as the HIV pandemic.[3] Current therapy (DOTS) consists of an initial phase with four drugs, isoniazid, rifampin, pyrazinamide and ethambutol daily for two months, followed by a continuation phase of treatment with isoniazid and rifampin thrice weekly for a further four months, and has a cure rate of up to 95%, given patient compliance.[4] Poor patient compliance with this prolonged regimen, together with other factors, has led to the emergence of multidrug-resistant tuberculosis (MDR-TB), against which DOTS is relatively ineffective.[5,6] In view of this, DOTS-Plus (DOTS plus second-line TB drugs) is now recommended for treating MDR-TB and TB in areas with high incidence of MDR-TB.[4] However, DOTS-Plus is expensive, takes longer to administer and has significant side-effects.[7]

Dihydrofolate reductase (DHFR) is an important enzyme in the folate cycle[8,9] which supplies one-carbon units derived from the action of serine hydroxymethyltransferase[10,11] on L-serine for the biosynthesis of deoxythymidine monophosphate (dTMP). Inhibition of the folate cycle leads to interruption of the supply of thymidine and thus to inhibition of DNA biosynthesis and inhibition of proliferation of cells. Inhibition of proliferation is a useful goal in the therapy of cancer[12] and of bacterial and protozoal infections.[13] Highly potent inhibition of DHFR

has been achieved with analogues of the substrate, dihydrofolate **1** (Figure 1). Methotrexate **2** is a highly potent inhibitor of mammalian DHFR and mammalian tumour DHFR (IC_{50} = 2.5 nM *vs*. rat liver DHFR)[14] and is one of the most widely used anticancer antimetabolite drugs. It has *ca*. seven-fold selectivity for inhibition of human DHFR *vs*. *M. tuberculosis* DHFR.[15]

The biological activities of pyrimidine-2,4-diamines have shown that it is not necessary to have the full pteridinediamine structure. These "non-classical" inhibitors have advantages in that they are more lipophilic than **2** and can enter cells by passive diffusion, not requiring the folate carrier. Pyrimethamine **3** was developed over 50 years ago as a DHFR-inhibiting anti-malarial drug;[16] it has selectivity for inhibition of *Plasmodium falciparum* DHFR activity of *ca.* forty-fold *vs*. human DHFR.[17] It is several orders of magnitude less potent than **2** against human DHFR.[17-19] Sulphadoxine / pyrimethamine plus isoniazid has some utility as prophylaxis against tuberculosis in HIV-positive pateints[20] but isoniazid itself has been implicated in inhibition of *M. tuberculosis* DHFR after metabolism.[21] DDMP / metoprine **4a** is a close analogue of **3** which shows a similar profile of inhibition of DHFRs, showing some activity as an antitumour agent in clinical trial.[22] However, this compound is also a highly potent inhibitor of histamine N-methyltransferase,[23,24] leading to neurological complications with its use. The 6-ethyl analogue etoprine **4b** shows similar antileukaemic activity;[25] its inhibition of testicular DHFR causes infertility in male rats.[26] Methylbenzoprim **5** was designed as a non-classical DHFR inhibitor which lacks the full pteridine ring structure of methotrexate **2** but remains extremely potent against mammalian DHFRs (IC_{50} *vs.* rat liver DHFR 3.2 pM) with some antitumour activity.[14] Interestingly, this compound is markedly less active against *Pneumocystis carinii*, *Toxoplasma gondii* and *Escherichia coli* DHFRs;[14,18] these activities have been rationalised in a crystallographic and modelling study.[18] Trimethoprim **6**, in which the 5-aryl substituent is linked through a methylene bridge for increased flexibility, is often cited as an inhibitor of *M. tuberculosis* DHFR and other bacterial DHFRs, yet it is reported to lack potency (IC_{50} 16.5 μM) and to be only five-fold selective for inhibition of *M. tuberculosis* DHFR *vs*. the human enzyme.[15] There is thus a great need for rationally designed selective inhibitors of *M. tuberculosis* DHFR for treatment of this widespread and often fatal disease.

2. Structure-based design

Several groups have pointed to structural differences between *M. tuberculosis* DHFR and human DHFR as possible opportunities for the design of selective inhibitors[15,27-29] but few studies have exploited these differences successfully in rational drug design for TB.[30] Da

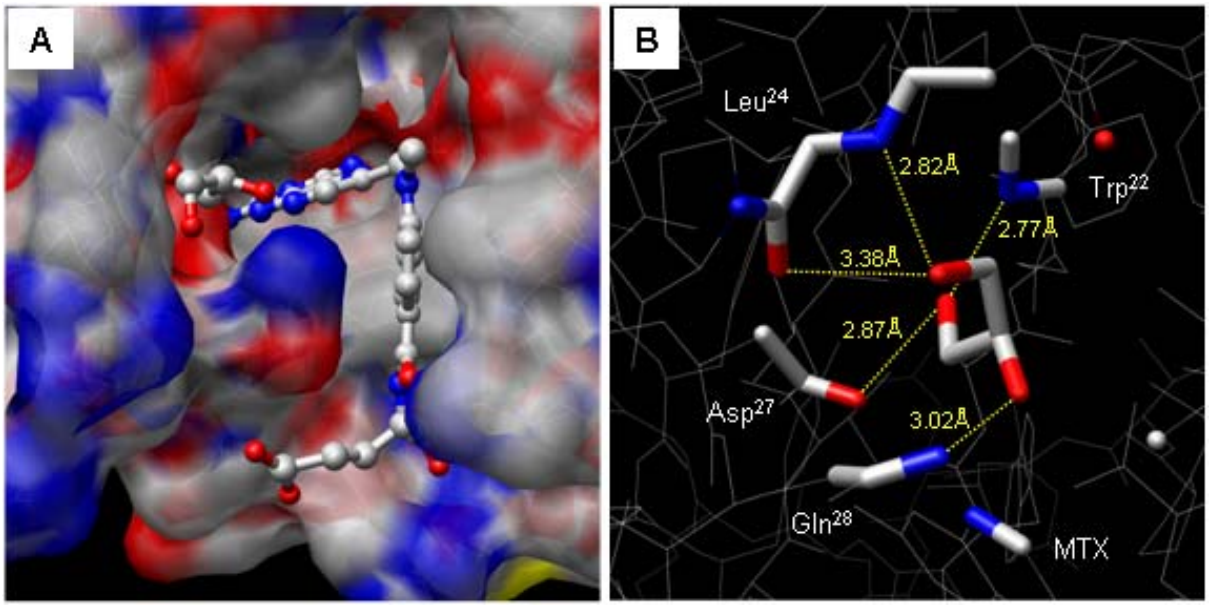
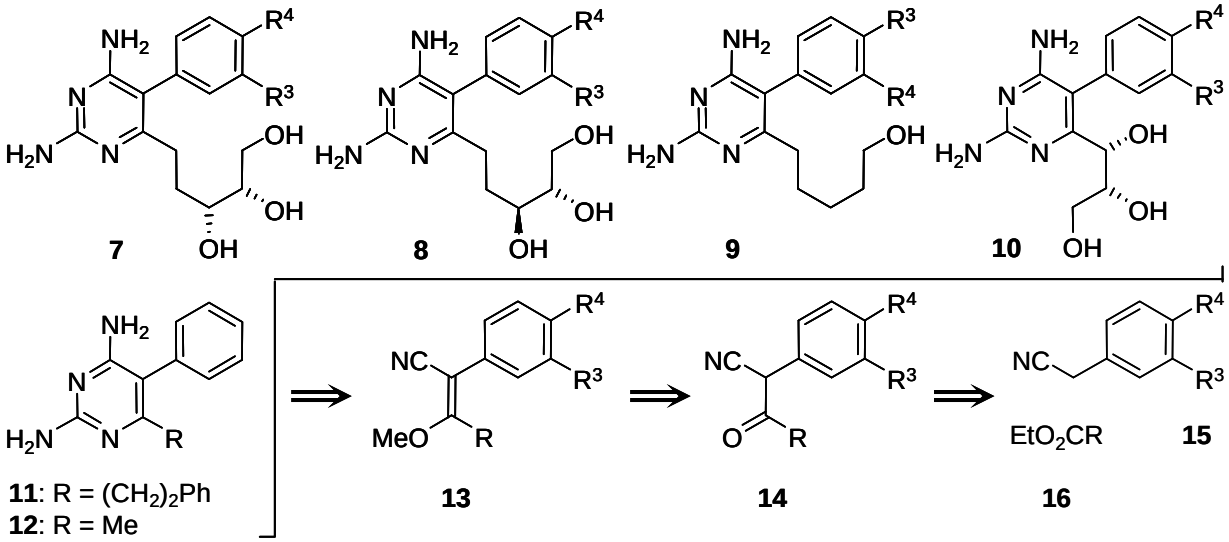


Figure 2. Images of structures of DHFR from *M. tuberculosis*, with methotrexate **2** bound at the dihydrofolate-binding site. **A**: View of the structure of *M. tuberculosis* DHFR with **2** bound, showing the glycerol molecule bound close to the active site (crystal structure reported by Li *et al.*[28]) (glycerol and **2** are shown as rods and balls; DHFR is shown as a surface with blue cationic, red anionic and grey hydrophobic neutral). **B**: Proposed H-bonds from the bound glycerol to the residues surrounding the glycerol pocket (atoms within 3.9 Å of the glycerol are shown as rods; other atoms and bonds are shown as wires).

Cunha *et al.*[30] have suggested that addition of hydrophobic groups to 5-deazapteridines should increase selectivity, based on six examples. Suling *et al.*[31] have achieved >100-fold selectivity for inhibition of the *M. avium* DHFR *vs*. human DHFR using similar 5-methyl-5-deazapteridine-2,4-diamines but have not published results for *M. tuberculosis* DHFR. Thus the way is open for rational structure-based design of selective inhibitors of *M. tuberculosis* DHFR exploiting a major difference between human and *M. tuberculosis* enzyme structures.

Li *et al.* reported crystal structures of *M. tuberculosis* DHFR. One structure contains methotrexate **2** bound at the dihydrofolate-binding site and $NADP^+$ at the $NADP^+$-binding site but also contains a glycerol tightly bound in an adjacent pocket where it forms H-bonds with Asp^{27}, Gln^{28} and Leu^{24} (Figure 2A).[28] This glycerol is also present in the structure of *M. tuberculosis* DHFR with the 1,3,5-triazine-2,4-diamine inhibitor Br-WR99210 bound but is absent from the crystal of *M. tuberculosis* DHFR containing **6**, probably owing to the fact that the trimethoxyphenyl unit causes the trimethoprim to bind in a different manner, causing the Gln^{28} side-chain to be disordered.[28] A more detailed examination of the environment of the glycerol reveals additional H-bonds (Figure 2B), as indicated by O—O and O—N distances and appropriate orientations. O(1)—H makes a H-bond with the side-chain amide carbonyl oxygen of Asp^{27}; O(1) is also involved as an acceptor in a H-bond with the indole N—H of



R = 3*R*,4*S*-3,4,5-trihydroxypentyl, 3*S*,4*S*-3,4,5-trihydroxypentyl, 5-hydroxypentyl, 1*S*,2*R*-1,2,3-trihydroxypropyl, $Ph(CH_2)_2$, Me
a: $R^3 = R^4 = H$; **b**: $R^3 = H$, $R^4 = Cl$; **c**: $R^3 = H$, $R^4 = Br$; **d**: $R^3 = R^4 = Cl$.

Scheme 1. Structures of designed pyrimidine-2,4-diamines **7**-**12** and retrosynthetic analysis.

Trp[22]. O(3) is also held in a two-H-bond clamp; O(3)—H makes a H-bond with the carbonyl oxygen of Leu[24] and is also an acceptor in a H-bond with the N—H of the same amino-acid. O(2) accepts a single H-bond from the side-chain amide N—H of Gln[28]. The glycerol carbon chain is in hydrophobic contact with Leu[20].[28] In contrast, in the structures of human DHFR complexes containing dihydrofolate or **2**, this site is well packed with hydrophobic side-chains.[32,33] Since this glycerol is clearly tightly and specifically bound in a fixed conformation close to N(8) of **2**, we designed series of molecules in which contain a 1,2,3-triol joined to a head group which would mimic the binding of **2** deep in the dihydrofolate-binding pocket.

Since **3** is a weak inhibitor of *M. avium* DHFR activity[34] and many other pyrimidine-2,4-diamines inhibit various DHFRs, we chose pyrimidine-2,4-diamine as the template to which to attach the linker from the triol. Compounds **7** (Scheme 1) were designed directly from modelling the orientation of the glycerol and overlay of the pyrimidine-2,4-diamine unit with the diaminopteridine of **2**. This overlay suggested that a two-carbon linker ($-CH_2CH_2-$) would be optimum to join the triol to the pyrimidine 6-position; it also showed the need for *R* configuration at the C(3) secondary alcohol of the 3,4,5-trihydroxypentyl side-chain (mimicking glycerol O(1)) and *S* configuration at the C(4) secondary alcohol (mimicking glycerol O(2)), as in **7**. The diastereomeric series **8** is *S* at C(3); this series tests the validity of the drug design, since the linker length is the same as in **7** but the orientation of the triol relative to the

pyrimidine-diamine should not be apposite for binding. In **9**, the secondary alcohols are missing, leaving only the primary alcohol of the 6-(5-hydroxypentyl) group to mimic O(3) of the glycerol and H-bond to Leu^{24} in the glycerol-binding pocket, losing the ability to H-bond to Trp^{22}, Asp^{27} and Gln^{28}, but retaining possible hydrophobic interactions with Leu^{20}. The length of the linker between the triol and the pyrimidine-2,4-diamine is tested in the 6-(1,2,3-trihydroxypropyl) compounds **10**; these compounds retain the triol motif with the same configuration at the secondary alcohols as in **7** but joined directly to pyrimidine C(6).

In each of the sets of 6-((poly)hydroxyalkyl)pyrimidine-2,4-diamines **7**-**10**, a phenyl is located at position-5 of the pyrimidine, to occupy a (largely) hydrophobic pocket which the hinge region (-CH_2NMe-) of **2** occupies in Figure 2A. This phenyl is unsubstituted in **7a**-**10a**, whereas this ring is halogenated in other designed compounds. It carries a 4'-chlorine in **7b**-**10b** (reflecting the 4'-chlorine in **3**) and a 4'-bromine in **7c**-**10c**. 3',4'-Dichlorophenyl was incorporated into **7d**, **9d** and **10d** to mimic the dichlorophenyl in **4a**,**b**; the corresponding analogue in the **8** series was planned but was synthetically inaccessible. Compounds **11** and **12** (Scheme 1) were designed as gross tests of the structure-based design of inhibitors, while retaining the essential pyrimidine-2,4-diamine. In **11**, the designed triol is replaced by a hydrophobic aromatic benzene ring which should interact unfavourably with the H-bonding environment of the glycerol-binding pocket. In **12**, there is no group which may enter this pocket.

3. Chemical synthesis

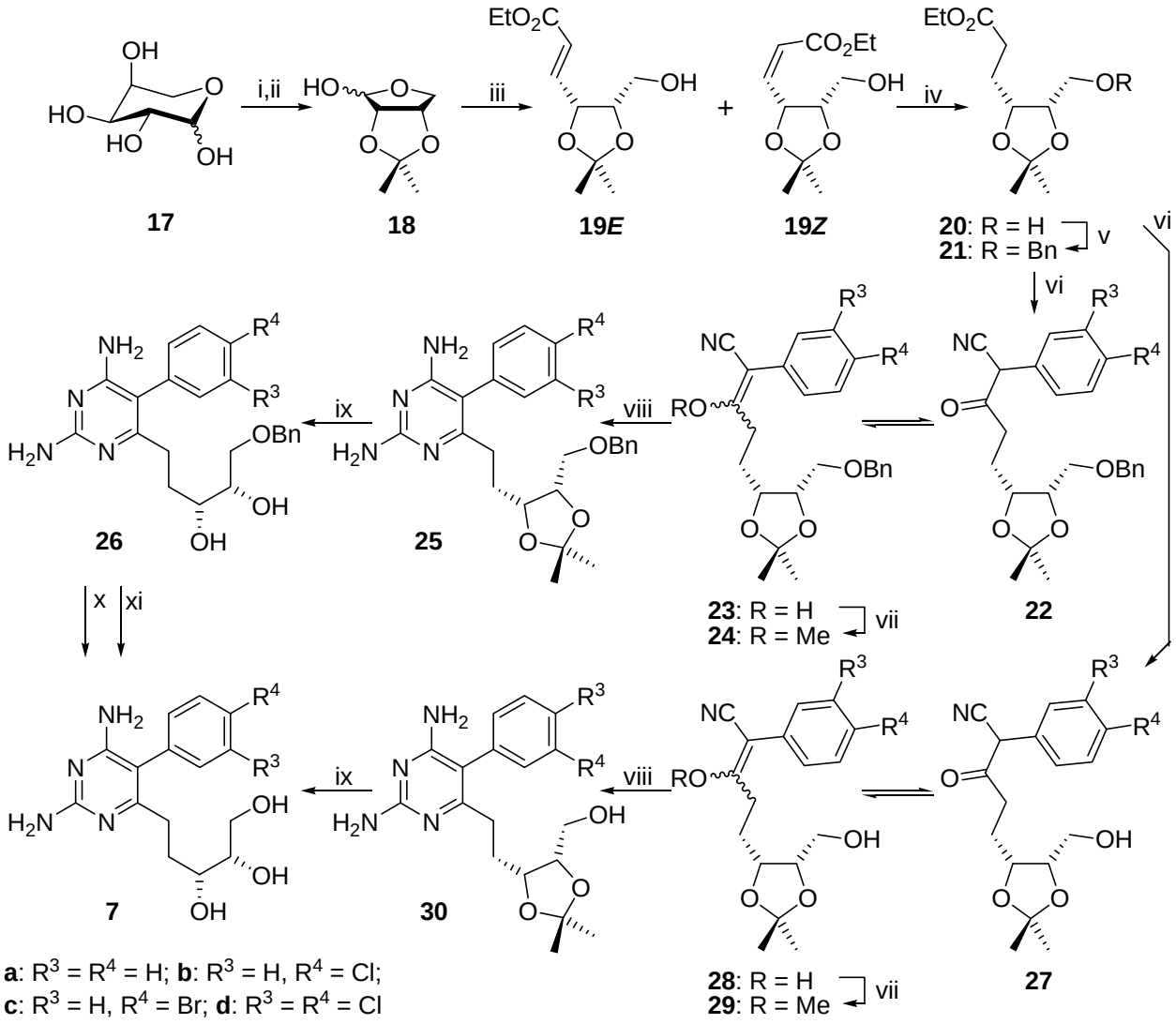
3.1. Synthetic strategy

The planned synthetic approaches to the series of target pyrimidine-2,4-diamines **7**-**12** are shown in retrosynthetic format in Scheme 1. In each case, condensation of an appropriately substituted corresponding enol ether **13** with guanidine would furnish the pyrimidinediamine. The enol ethers would be readily prepared by methylation of the α-acylphenylacetonitriles **14**, which, in turn would be available by acylation of anions derived from (Ar-substituted)phenylacetonitriles **15** with the appropriate esters **16**, with or without protection of the side-cahin alcohols. Several questions needed to be addressed during the development of the synthetic routes: how should the condensation with guanidine be optimised? how should the acylation be optimised? do the primary and secondary alcohols in the side-chains need to be protected during the acylation or condensation steps? if so, what should the protecting groups be? We elected to use the general synthetic approach, condensation of guanidine with enol ethers

derived from α-acylphenylacetonitriles, used by Russell and Hitchings[16] in their syntheses of pyrimethamine **3**, etoprine **4** and related antimalarial compounds carrying simple small-alkyl substituents at the 6-position of the pyrimidine-2,4-diamine core. Tarnchompoo *et al.*[19] have extended this synthetic approach to analogues carrying larger alkyl and ω-arylalkyl groups at this position, in their search for pyrimidine-2,4-diamines which inhibit DHFR activity in *Plasmodium falciparum* which is resistant to **3**. The acylation steps and the protection of the OH groups were optimised individually for each series of target compounds.

3.2. Synthesis of 5-aryl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines 7

Scheme 2 shows our approach to the 5-aryl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines **7**, using protection for the primary alcohol. We rationalised that the ester **21** would provide the required masked triol at the 6-position and could be synthesised by a two-carbon chain extension from a protected L-erythrose **18**. Acetonide protection was introduced between the *cis* 3-OH and 4-OH of L-arabinose **17** by acid-catalysed reaction with 2,2-dimethoxypropane. Oxidative cleavage of the C(1)—C(2) bond with periodate then gave L-erythrose-2,3-acetonide **18**. The required two-carbon chain-extension was achieved by base-free Wittig reaction of the latent aldehyde of **18** with pre-formed ethyl triphenyl-phosphoranylidineacetate to afford the stereoisomeric α,β-unsaturated esters **19*E*** and **19*Z*** in 69% overall yield (ratio of geometrical isomers 3:11, **19*E*** and **19*Z***, respectively). These geometrical isomers were readily separated chromatographically and were identified on the basis of the ^{1}H NMR coupling constants in the –HC=CH- system. Separation of the isomers was unnecessary in the synthetic plan, as catalytic hydrogenation of the mixture of **19*E*** and **19*Z*** gave the saturated ester **20** quantitatively. ^{1}H NMR spectroscopy confirmed the presence of only one diastereoisomer of **20**. A variety of protecting groups was investigated for the primary alcohol; we proposed that this alcohol should not be exposed during the reaction of the ester with the carbanion derived from the phenylacetonitriles, to avoid possible quenching of the carbanion and to avoid lactonisation of the hydroxy-ester **20**. The primary alcohol of **20** was benzylated by generation of the alkoxide with lithium bis(trimethylsilyl)amide and reaction with benzyl bromide to give the fully protected ester **21**. The classical conditions for using esters to acylate phenylacetonitrile carbanions,[16] sodium ethoxide in ethanol, failed to effect the required reaction. However, the carbanions were generated from the (halo)phenylacetonitriles under aprotic conditions with lithium bis(trimethylsilyl)amide in diethyl ether at low temperature; these reacted with **21** to afford the α-acylphenylacetonitriles **22a-d** in 16-26%



Scheme 2. Synthetic routes to 5-aryl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines **7**, using Bn protection for the primary OH and omitting protection for the primary OH. *Reagents*: i, $Me_2C(OMe)_2$, TsOH, DMF; ii, $NaIO_4$, H_2O, hexane; iii, $EtO_2CCH{=}PPh_3$, CH_2Cl_2; iv, H_2, Pd/C, EtOH; v, $LiN(SiMe_3)_2$, BnBr, THF, DMF; vi, $LiN(SiMe_3)_2$, $ArCH_2CN$, Et_2O; vii, CH_2N_2, Et_2O; viii, guanidine.HCl, NaOMe, $MeO(CH_2)_2OH$; ix, aq. CF_3CO_2H; x, Na, liquid NH_3, xi, $FeCl_3$, CH_2Cl_2.

yields. The ^{1}H NMR spectra indicated the presence of varying amounts of the enol tautomers **23a-d**. Methylation with diazomethane gave the enol ethers **24a-d** as inseparable mixtures of geometrical isomers. Condensation of these mixtures with guanidine in boiling 2-methoxy-ethanol then led to the pyrimidine-2,4-diamines **25a-d** in satisfactory yields; similar reactions in the conventional solvent for these condensations, ethanol, gave lower yields.

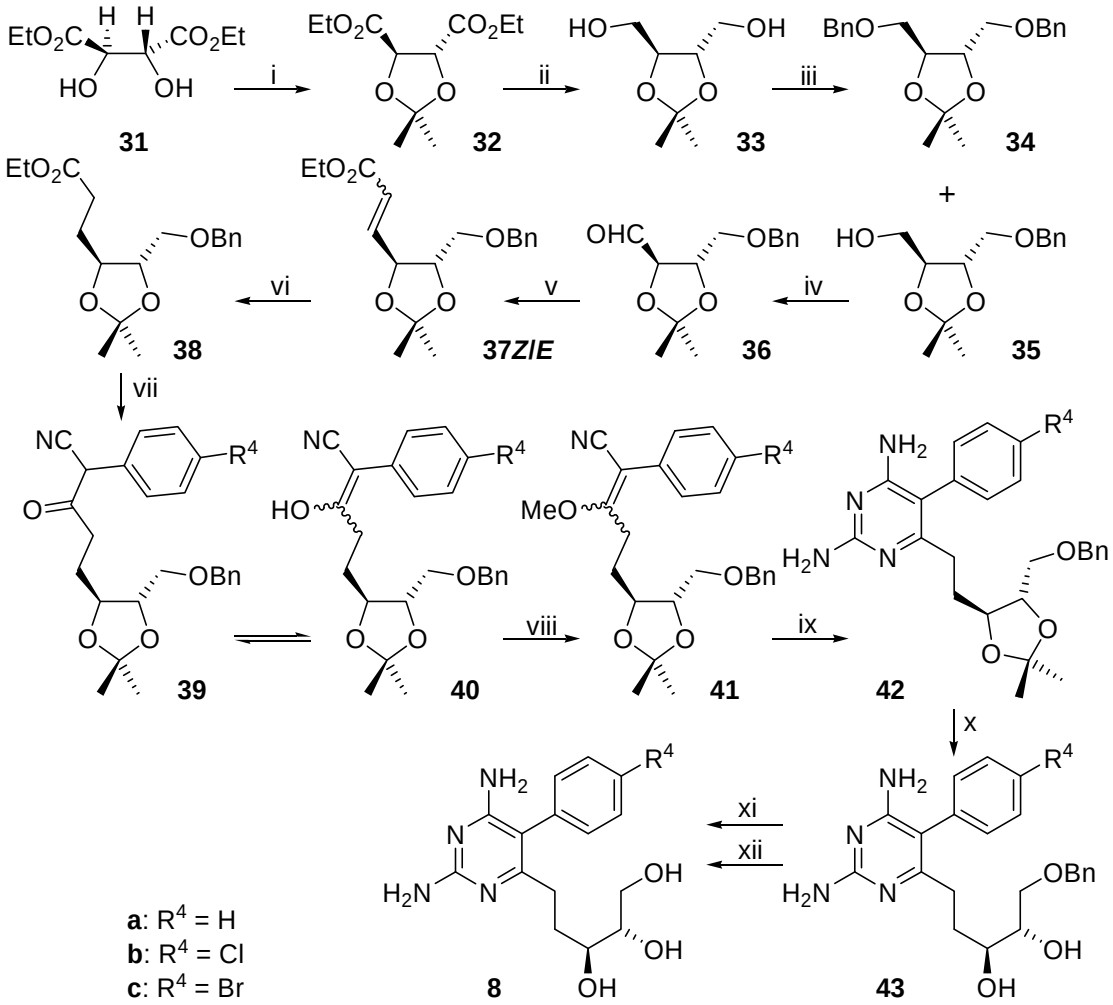
Removal of the acetonide protection from **25a-d** with aq. trifluoroacetic acid revealed the secondary alcohols in **26a-d** in excellent yields but subsequent removal of the benzyl protection from the primary alcohol was more challenging. Catalytic hydrogenolysis (H_2, Pd/C, various solvents) failed to remove the benzyl group from **26a**, even in the presence of catalytic per-

chloric acid. However, addition of a catalytic amount of chloroform[35] to the hydrogenolysis reaction mixture in methanol facilitated the deprotection to give triol **7a**. This method could not be extended to debenzylation of the halogen-bearing analogues **26b-d**, as hydrogenolysis of the carbon—halogen bonds occurred; **26b** and **26c** gave **7a** only, whereas **26d** gave an inseparable mixture of **7a**, **7b** and the *meta*-monochloro analogue. Attempted debenzylation with hydrogen bromide in acetic acid, another common method, gave regioisomeric mixtures of bromo- and acetoxy-pentylpyrimidine-2,4-diamines. The most effective method for preparation of the Ar-unsubstituted analogue **7a** was reductive cleavage of the O—benzyl protecting group with sodium in liquid ammonia. This method could not be extended to preparation of the halogenated congeners **7b-d**, as reduction of the carbon—halogen bonds led to exclusive formation of the phenyl analogue **7a** from **26b-d**. The most generally applicable debenzylation for this series was the use of the Lewis acid anhydrous iron(III) chloride in dichloromethane, as developed by Park *et al*.[36] By this method, **26b-d** were converted in high yields into the required triols **7b-d**. Moreover, the Lewis acidity of this reagent could be exploited also in removal of the acetonides, in that both acetonide and benzyl ether protecting groups could be removed from **25a-d** in one pot to furnish **7a-d** directly, albeit in lower overall yields than in the two-step processes. TLC analysis suggested that, in this one-pot process, the acetonide was cleaved within 5 min and the debenzylation was essentially complete within 80 min.

In view of these challenges, the assembly of the pyrimidine ring was attempted with a free primary alcohol in the side chain. As shown in Scheme 2, the phenylacetonitriles were deprotonated with lithium bis(trimethylsilyl)amide and the anions were quenched with the ester **20**. Use of two equivalents of base was necessary to achieve condensation to obtain the α-acylphenylacetonitriles **27** in a maximum yield of 10%, indicating that protection of the primary alcohol is beneficial for this acylation to proceed efficiently. Methylation of the tautomeric enols **28** with diazomethane and condensation of the enol ethers **29** with guanidine gave the pyrimidine-2,4-diamines **30**. Again, the yields were significantly lower with the exposed primary alcohol (**30a**: 42%, **30b**: 12%, **30c**: 20%, **30d**: 9%). Deprotection was straightforward to furnish the target triols **7**.

3.3. Synthesis of 5-aryl-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines 8

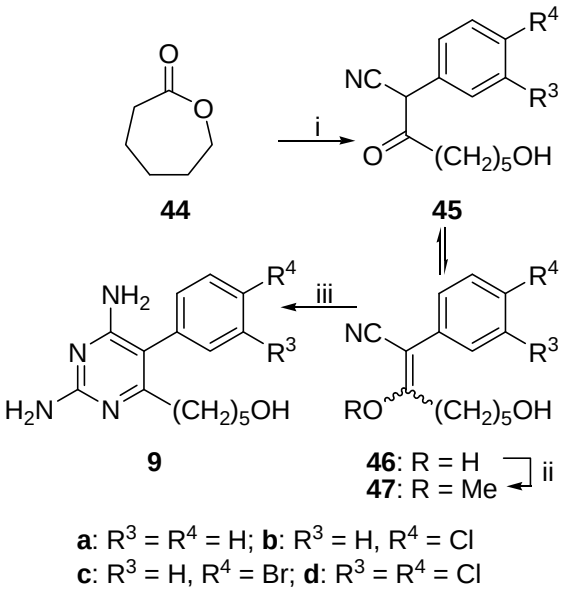
The approach to the diastereomeric 5-aryl-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines **8** was broadly similar to that for **7**, using the benzyl protection method. In this series (Scheme 3), the key intermediate was the *trans* dioxolane **38**, a diastereomer of the *cis*



Scheme 3. Synthesis of 5-aryl-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines **8**. *Reagents*: i, $Me_2C(OMe)_2$, TsOH, 4 Å molecular sieve, CH_2Cl_2; ii, $LiAlH_4$, THF; iii, NaH, BnCl, DMF; iv, PCC, NaOAc, 4 Å molecular sieve, CH_2Cl_2; v, $EtO_2CCH{=}PPh_3$, $PhCO_2H$, PhMe, Δ; vi, H_2, Pd/C, EtOH; vii, $LiN(SiMe_3)_2$, $ArCH_2CN$, Et_2O; viii, CH_2N_2, Et_2O; ix, guanidine.HCl, NaOMe, $MeO(CH_2)_2OH$; x, aq. CF_3CO_2H; xi, H_2, Pd/C, EtOH; xii, $FeCl_3$, CH_2Cl_2.

dioxolane ester **21** above. The approach to **38** started with protection of the secondary alcohols of diethyl *R*,*R*-tartrate **31** as the acetonide **32**; these secondary alcohols will become the secondary alcohols of the targets **8** with the appropriate configurations. Reduction with lithium aluminium hydride furnished the C_2-symmetric diol **33**. Mono-protection of this diol was essential for developing the chain-extension of only one arm. The optimum conditions were found to be deprotonation with one equivalent of sodium hydride in DMF, followed by alkylation with benzyl chloride, giving the required monoether **35**, with a trace of diether **34**. Pyridinium chlorochromate oxidation converted the exposed alcohol to the aldehyde **36**, which was immediately condensed with ethyl triphenylphosphoranylidineacetate in a Wittig reaction to give the chain-extended α,β-unsaturated esters **37*E*** and **37*Z***. In contrast to the analogous uncatalysed formation of **19*E*** and **19*Z*** (which carry free primary alcohols) at ambient temper-

ature, this reaction required prolonged heating at 110°C and catalysis with benzoic acid. In this case, the mixture of the separable geometrical isomers **37*E*** and **37*Z*** was approximately equimolar. Careful control of the hydrogenation conditions was required to reduce the alkene of the **37*E*** / **37*Z*** mixture to form key intermediate **38** without causing loss of the benzyl protecting group through hydrogenolysis. The fully protected ester **38** was then used, as for the diastereomer **21**, to alkylate the carbanions derived from the (halo)phenylacetonitriles to afford the α-acylphenylacetonitriles **39a-c**; 3,4-dichlorophenylacetonitrile failed to react. Methylation of the enols **40** and condensation of the enol ethers **41** with guanidine led to the pyrimidine-2,4-diamines **42**, in much higher yields (**42a**: 67%, **42b**: 48%, **42c**: 53%) than in the *R,S* series. The side-chain alcohols were deprotected in two steps. Acid-hydrolysis of the acetonide rapidly gave the diols **43**. As in the diastereomeric series, hydrogenolysis removed the benzyl group from **43a** to afford **8a** in high yield; debenzylation with iron(III) chloride converted **43b** and **43c** to the triols **8b** and **8c**, respectively, avoiding the dehalogenations associated with other debenzylation procedures.



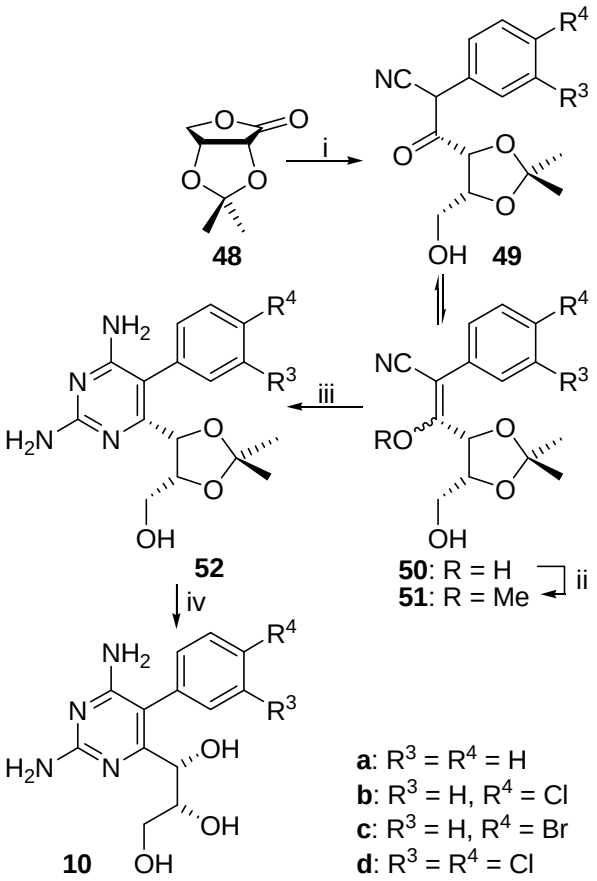
Scheme 4. Synthesis of 5-aryl-6-(5-hydroxypentyl)-pyrimidine-2,4-diamines **9**. *Reagents*: i, $LiN(SiMe_3)_2$, $ArCH_2CN$, Et_2O; ii, CH_2N_2, Et_2O; iii, guanidine.HCl, NaOMe, $MeO(CH_2)_2OH$.

3.4. Synthesis of 5-aryl-6-(5-hydroxypentyl)pyrimidine-2,4-diamines 9

Although a similar approach of protection of the primary alcohol could have been used in the syntheses of 6-(5-hydroxypentyl)pyrimidine-2,4-diamines **9**, a strategy was devised to use a lactone to provide the necessary acylating ester, simultaneously masking the primary alcohol (Scheme 4). The carbanions of the (halo)phenylacetonitriles were generated in the usual way with lithium bis(trimethylsilyl)amide; the yields of the reactions with lactone **44** to give the α-(6-hydroxyhexanoyl)phenylacetonitriles **45** were low but provided sufficient material for further methylation of the enols **46** and condensation of **47** with guanidine to give the required 6-(5-hydroxypentyl)pyrimidines **9** in moderate yields. No deprotection steps were required in this series as the primary alcohols had been revealed during the reaction of the lactone with the phenylacetonitrile anions.

3.5. Synthesis of 5-aryl-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamines 10

The lactone strategy was also used for the chain-shortened triols **10** (Scheme 5). 2,3-*O*-Isopropylidene-D-erythronolactone **48** reacted with the phenylacetonitriles anions to afford **49** in 21-38% yields. In the usual way, methylation of the enols **50**, condensations of the enol ethers **51** with guanidine and aqueous acid deprotection of **52** gave the pyrimidine-2,4-diamines **10** carrying the 6-((1*S*,2*R*)-1,2,3-trihydroxypropyl) side-chains.



Scheme 5. Synthesis of 5-aryl-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamines **10**. *Reagents*: i, $LiN(SiMe_3)_2$, $ArCH_2CN$, Et_2O; ii, CH_2N_2, Et_2O; iii, guanidine.HCl, NaOMe, $MeO(CH_2)_2OH$; iv, aq. CF_3CO_2H.

The dioxolanylpyrimidine intermediates **52** carry two bulky groups in close proximity in the 5- and 6-positions of the pyrimidine. MM2 energy minimisation suggests that this twists the 5-(4-halo)phenyl group in **52a-c** out of the pyrimidine plane by *ca*. 60° (Figure 3). The restricted rotation about the pyrimidine—Ph bond is evident in the NMR spectra of these compounds. The benzene ring is held close to the dioxolane, which bears two chiral centres. Thus the Ph 2-H and 6-H become diastereotopic, as do the Ph 3-H and 5-H. For example, in the ^{1}H NMR spectrum of **52a**, the Ph 2-H signal is separated from the Ph 6-H signal by 0.21 ppm, whereas the 3-H and 5-H signals are coincident. In the spectrum of the 4-chloro compound **52b**, the Ph 2-H and 6-H signals are separated by 0.22 ppm and the 3-H and 5-H signals are separated by 0.04 ppm. In the spectrum of the 4-bromo compound **52c**, the separations are 0.05 ppm and 0.02 ppm, respectively. In **52a-c**, the substituents, if present, are in the 4-position of the benzene ring and are therefore coaxial with the pyrimidine—benzene bond. However, **52d** carries a chlorine atom in position-3 of the benzene ring, which is off the axis of this bond. Therefore, two different conformers **52dA** and **52dB** can exist, as shown in Figure 3 in stick and space-filling representations. Conformers **52dA** and **52dB** are diastereoisomers of very similar energy,

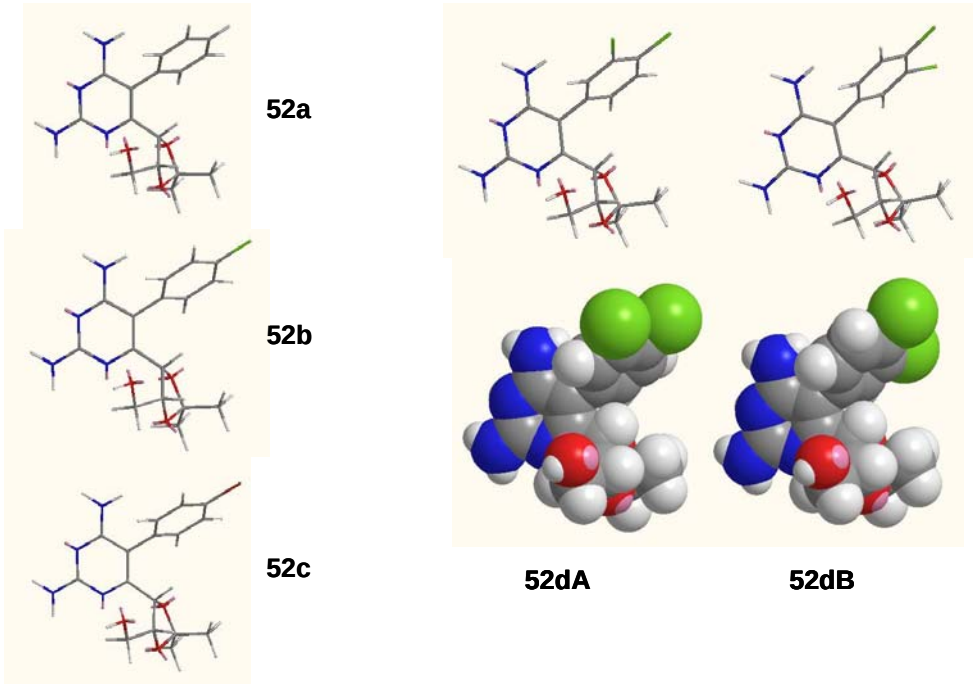
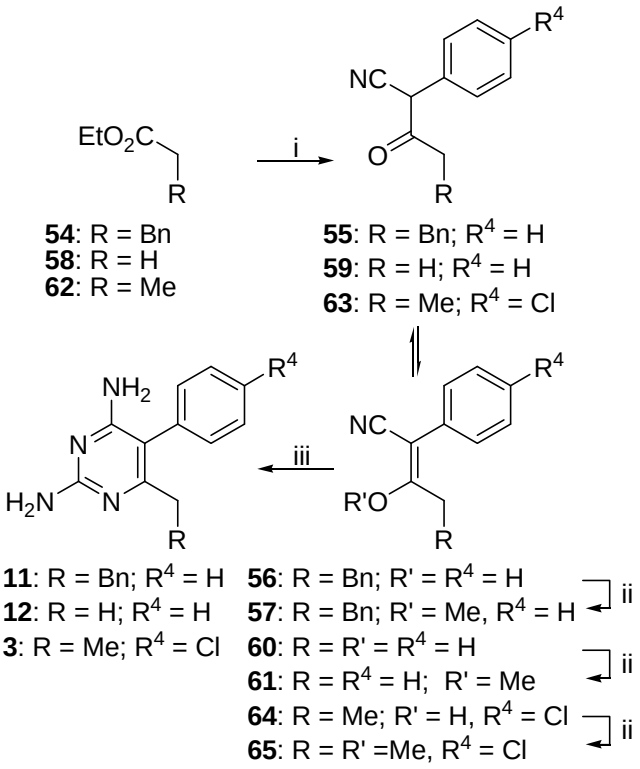


Figure 3. MM2-minimised structures of pyrimidine-2,4-diamines **52a-d**, showing the steric interactions between the 5-(halo)phenyl group and the 6-(2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl) substituent. As a result of this steric crowding, the (halo)phenyl group is twisted to *ca.* 60° from the plane of the pyrimidine. Compound **52d** exists as two diastereomeric conformers, which are evident in the ^{1}H and ^{13}C NMR spectra.

according to MM2 calculations. The ^{1}H NMR spectrum of **52d** shows the presence of both conformers in 1:1 ratio; the sharpness of the signals indicates that, as could be predicted from the severe steric crowding, interconversion is slow. The ^{1}H signals for 2-H for the diastereomeric conformers are separated by 0.10 ppm, the signals for 5-H by 0.02 ppm and the signals for 6-H by 0.10 ppm. Other ^{1}H NMR signals are co-incident for the two conformers, as are all the peaks in the ^{13}C NMR spectrum. The latter was assigned by analogy with the spectra for **3** and related compounds examined in detail earlier.[37] This effect was not observed for the triols **10a-d** and only one set of signals could be seen for each compound, with 2-H and 6-H being magnetically equivalent. This probably reflects the greater flexibility in the triol side-chain. The effects were also not observed for the homologues **7** and **8**, also owing to increased flexibility and the remoteness of the chiral dioxolane from the benzene ring in these structures.

3.5. Synthesis of pyrimidine-2,4-diamines 11, 12 and 3, lacking OH in the 6-substituent

Three pyrimidine-2,4-diamines **11**, **12** and **3**, lacking alcohols in the 6-substituent, were required as controls in the biological evaluation. The synthetic approaches followed the general sequence (Scheme 6). Acylation of phenylacetonitrile anion with ethyl 2-phenylpropanoate **53**, methylation of **55** and condensation of **56** with guanidine gave 6-(2-phenylethyl)pyrimidine-2,4-diamine **11**. The minimal analogue **12** was prepared similarly, through acylation of phenylacetonitrile anion with ethyl acetate **57**, methylation of **59** and condensation of **60** with guanidine. Finally, **3** was produced in a new route starting with generation of the anion from 4-chloroacetonitrile with $LiN(SiMe_3)_2$ and reaction with ethyl propanoate **61** to give **62**. Enol **63** was methylated, giving **64**; condensation with guanidine in hot 2-methoxyethanol provided **3** in good yield.



Scheme 6. Synthesis of 5-phenyl-6-(2-phenylethyl)-pyrimidine-2,4-diamine **11**, 6-methyl-5-phenylpyrimidine-2,4-diamine **12** and pyrimethamine **3**. *Reagents*: i, $LiN(SiMe_3)_2$, $ArCH_2CN$, Et_2O; ii, CH_2N_2, Et_2O; iii, guanidine.HCl, NaOMe, $MeO(CH_2)_2OH$.

4. Biological evaluation

4.1. Inhibition of DHFR activities

Direct screening of candidate drugs with *M. tuberculosis* is slow and requires biosafety Level 3 facilities and procedures.[38] The slow growth of *M. tuberculosis* has been frustrating, with most public health laboratories still employing cultivation techniques that require 3-6 weeks to achieve growth. This mainly reflects the slow generation time inherent in the organism. *M. smegmatis* and *M. avium* have often been used as surrogates for assessment of activity of candidate drugs, as they grow rapidly and are less pathogenic to humans.[39-41] However, drug screening in wild-type *M. smegmatis* has not always been an accurate predictor of activity[42] or of mechanism of action in *M. tuberculosis*.[43]

A new approach to screening compounds for selective inhibition of DHFR from *M. tuberculosis* has been developed by Gerum *et al*.[38] In this, the TH5 strain of the yeast *Saccharomyces cerevisiae*, which lacks endogenous expression of DHFR, was engineered to contain a vector p414CYC1 carrying a single copy of the *dfrA* gene from *M. tuberculosis*. This gene codes for the protein with DHFR activity in *M. tuberculosis*. The native TH5 strain of *S. cerevisiae* requires supplementation with dTMP, uracil, adenine and a full complement of amino acids to grow, whereas the engineered strain containing the *dfrA* gene can grow normally. Thus inhibition of the expressed *M. tuberculosis* DHFR activity would be manifest as inhibition of growth of the yeast. Two engineered TH5-derived strains of *S. cerevisiae* were also engineered to carry yeast or human DHFR genes. Inhibition of the growth of these yeasts by test compounds would indicate that these eukaryotic DHFRs are inhibited and would point to lack of selectivity for the prokaryotic *M. tuberculosis* enzyme. These three engineered yeasts were kindly supplied by Dr. Carol Hopkins Sibley (Department of Genome Sciences, University of Washington, Seattle, Washington, USA). Thus, in the present work, the test compounds were evaluated for their ability to inhibit selectively the growth of yeast carrying *M. tuberculosis* DHFR, while having less inhibition of yeast bearing either the yeast or the human enzyme. This assay, performed on a spoke assay plate, is semi-quantitative; comparison of the diameters of the zones of inhibition of the three yeasts by a particular test compound gives an indication of the selectivity of inhibition of the *M. tuberculosis* DHFR by that compound. Compounds can also be ranked approximately for potency of inhibition, although no quantitative IC_{50} data can be derived.

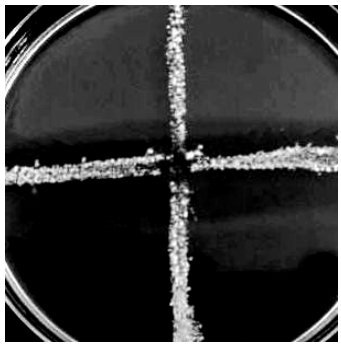


Figure 4. Typical plate (DMSO only control) showing orthogonal streaks of yeast after 3 d of incubation.

Table 1 shows the mean diameters of the zones of inhibition of growth of the three yeasts by the pyrimidine-2,4-diamines **7**-**10** carrying one or more alcohols in the side-chain, by the pyrimidine-2,4-diamines **11** and **12** with simple lipophilic side-chains and by the known DHFR-inhibiting pyrimidine-2,4-diamines **3** and **6**. Data for the negative control, DMSO without drug, are also given. Trimethoprim **6** has been reported to have a broad spectrum of activity against gram-positive bacteria, including methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus*, and gram negative bacteria, including *E. coli*, but less activity or no activity against *Mycobacterium spp.*, *Ps. aeruginosa* and *Chlamydia pneumoniae*.[44]

At the enzymic level, **6** is only a weak inhibitor of *M. avium* DHFR and of eukaryotic DHFR but is potent in inhibiting DHFR activity in susceptible bacteria.[41] In line with these reports, **6** was found to be inactive against all three DHFRs in this yeast assay. Pyrimethamine **3** was very poorly active, even against the human enzyme, despite being reported to have K_i = 58 nM against human DHFR.[17] This observation suggests that, despite being only semi-quantitative, the assay is a stringent test of inhibitory activity. The pyrimidine-2,4-diamine **11**, which lacks hydroxy groups and carries only lipophilic substituents was also inactive against all the DHFRs. Interestingly, the minimal lipophilic pyrimidine-2,4-diamine **12**, which bears only a methyl group at position-6, showed some inhibitory activity, although it was unselective.

Pyrimidine-2,4-diamines **7a-d**, which carry the (3*R*,4*S*)-3,4,5-trihydroxypentyl side-chain at the 6-position were designed to mimic directly the pteridine of the dihydrofolate and the glycerol, with the configuration of each chiral centre being as predicted by the structure-based design; the $–CH_2CH_2-$ linker is also of the length indicated by the modelling studies to be apposite. Within this set, the 5-phenyl compound **7a** showed notable selectivity for inhibition of the growth of the yeast containing the *M. tuberculosis* DHFR, with only very modest inhibition of the growth of the yeasts containing the *H. sapiens* enzyme or the *S. cerevisiae* enzyme. The 4'-chlorophenyl analogue **7b** also showed some selectivity for inhibition of the *M. tuberculosis* enzyme, whereas the 4'-bromophenyl and 3',4'-dichlorophenyl compounds **7c** and **7d** had modest and equivalent activity against each DHFR.

The diastereomeric series **8a-c** showed modest activity but little evidence of selectivity. Removal of the secondary alcohols from the 6-position side-chain, in **9**, led to compounds with increased potency but completely lacking selectivity. In contrast, shortening the side-chain by removal of the $–CH_2CH_2-$ linker but retaining the configuration of the secondary alcohols effectively abolished inhibitory activity in **9a-c** but the 3',4'-dichlorophenyl compound **9d** showed modest but non-selective inhibition of all the DHFRs.

Several trends are noticeable in the structure-activity relationships for these pyrimidine-2,4-diamines. Firstly, comparison of the results for **7a**,**b** with those for the diastereoisomers **8a**,**b** indicates that the configuration of the hydroxy groups is critical for selective inhibition of *M. tuberculosis* DHFR, as predicted by the model. Secondly, the secondary alcohols appear to be necessary to use the binding contacts of one primary and the secondary alcohol of the glycerol, in that the 6-(5-hydroxypentyl) compounds **9** are not selective for the *M. tuberculosis* enz-

yme. Thirdly, the length of the linker joining the dihydrofolate mimic (the pyrimidine-2,4-diamine) to the glycerol mimic is critical; shortening the distance in **10** abolishes activity.

4.2. Modelling of the selective inhibitor 7a in the dihydrofolate- and glycerol-binding sites of *M. tuberculosis* DHFR

The structures of selected pyrimidines from the series were modelled into the dihydrofolate-binding site and the glycerol pocket, to attempt to rationalise the structure-activity observations and thus to validate the design process. The compounds were bound into the dihydrofolate and glycerol binding pockets using the H-bonds from the pyrimidine-2,4-diamine ring to establish an orientation similar to the observed binding conformation of methotrexate **2**.[28] The triol section was then docked using the H-bonds established from the bound glycerol in the X-ray structure (as distance restraints). Molecular dynamics calculations were then performed on the bound ligand using the H-bonds (X-ray observed) as distance restraints between the bound ligand and the pocket. The ligand was ramped to 300 K over a period of 10 ps and then held at 300 K for 20 ps. Observing the conformations over the final 20 ps gave two distinct binding conformers. Throughout the above procedure, the binding pocket was restrained and only the ligand was allowed to change orientation. Average structures were taken (7-13 ps and 15-20 ps) which were then minimised within a restrained binding pocket. The two structures obtained were then freely minimised (ligand and binding pocket to a radius of 15 Å) to give the structures and conformations shown in Figure 5.

Figure 5 shows the occupation of these sites by the two conformers of **7a**, the most selective inhibitor of *M. tuberculosis* DHFR. As expected, the triol makes H-bonds with Asp^{27}, Gln^{28}, Leu^{24} and Trp^{22}, following the pattern shown by the glycerol in the crystal structure.[28] With the glycerol-mimicking triol held by the hydrogen-bonding network, the pyrimidine-2,4-diamine is perfectly located for its own hydrogen-bonding interactions deep in the dihydrofolate-binding site. These constraints place the 5-phenyl substituent of **7a** in a pocket of limited size. Indeed, this pocket cannot accomodate halogens in the 4'-position of the phenyl, as this position is tight against the surface of the enzyme; thus the observations that the 4'-bromo- and 3',4'-dichloro- analogues (**7c** and **7d**, respectively) not selective inhibitors are rationalised in the model. The 4'-chloro- analogue **7b**, however, does show slight selective inhibition of *M. tuberculosis* DHFR and it may be possible to accommodate the chlorine, albeit with a significant penalty in displacing the other binding contacts from their ideal positions.

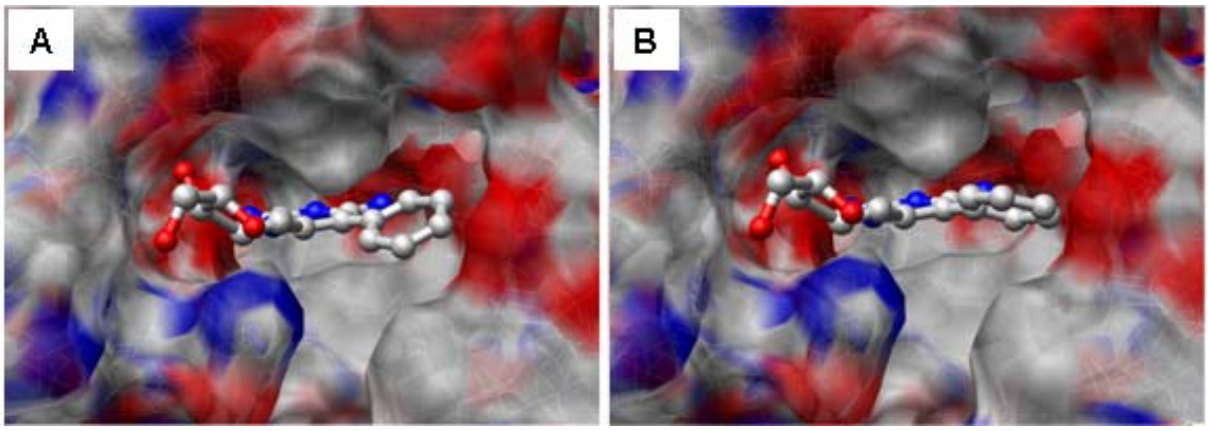


Figure 5. Images of structures of DHFR from *M. tuberculosis*, with **7a** bound at the dihydrofolate-binding site (structure derived from modelling study, see text). **A**: View of the structure of *M. tuberculosis* DHFR with **7a** bound in conformation **7aA**; **B**: View of the structure of *M. tuberculosis* DHFR with **7a** bound in conformation **7aB**.

The active lead compound **7a** can adopt two different conformations. As with all 5-(substituted)phenyl-6-substituted-pyrimidine-2,4-diamines, the 5-phenyl ring of **7a** has to be twisted out of the plane of the aromatic heterocycle to accommodate the adverse steric interactions between the phenyl *ortho*-hydrogens and the adjacent 4-NH_2 and 6-substituent. This rotation about the Ph—pyrimidine bond can be either clockwise or anticlockwise to achieve the same relief of steric strain. In conformer **7aA**, the phenyl is rotated anticlockwise from coplanarity, whereas clockwise rotation produces **7aB**; these conformers are almost identical in energy in free space. However, **7aA** fits well into the pocket in the *M. tuberculosis* DHFR (Figure 5A), whereas the forward edge of the 5-phenyl of **7aB** is located tightly pressed against the top of the enzyme pocket (Figure 5B). Thus the calculated energy of the complex of *M. tuberculosis* DHFR with conformer **7aA** is of consistently higher energy than than of the complex of *M. tuberculosis* DHFR with conformer **7aB**; indicating that **7a** binds in conformer **7aA**.

5. Conclusions

In this paper, we have reported our exploitation of a major difference in the local structure in the region of the dihydrofolate-binding sites of human and *M. tuberculosis* DHFR to design a compound **7a** which shows notable selectivity for inhibition of the latter. In the crystal structure of a *M. tuberculosis* DHFR ternary complex with methotrexate **2** and glycerol, the glycerol is held tightly in its binding pocket by a network of five H-bonds. This glycerol-binding pocket is close to the site of the methotrexate. This glycerol-binding pocket is absent from the structure of human DHFR. In the structures of **7**, the two-carbon link suggested by the crystal structure joins a triol (mimicking the glycerol) to the 6-position of a pyrimidine-

2,4-diamine core which binds into the dihydrofolate-binding site. The configurations of the secondary alcohols match the orientation of the glycerol relative to the methotrexate in the crystal structure. Three series of analogues were also designed to test the hypotheses of the design of **7**. Compounds **8** tested the assignment of the configuration of the point of attachment of the triol to the linker and hence to the pyrimidine-2,4-diamine. Mono-hydroxy compounds **9** tested the need to take up the H-bonds from all three alcohols of the glycerol in binding selectively to the mycobacterial DHFR. Compounds **10** tested the length of the linker between the triol moiety and the pyrimidine-2,4-diamine.

The target compounds were synthesised by acylation of the anions derived from phenylacetonitriles with appropriately functionalised and protected esters and lactones, followed by methylation, condensation with guanidine and deprotection, if appropriate. The acylation step was optimised as generation of the phenylacetonitrile anion with lithium bis(trimethylsilyl)-amide at -78°C, followed by addition of the ester or lactone. Yields under these optimised conditions ranged from 6% to 41%, with the lower yields being obtained with substrates containing unprotected alcohols. The condensations with guanidine were generally uneventful and high yielding. Removal of benzyl groups presented a particular challenge, as many reductive methods also effected dehalogenation in some analogues.

Evaluation of the test 6-substituted pyrimidine-2,4-diamines for their inhibition of the growth of yeasts containing active DHFR from human, *M. tuberculosis* and yeast indicated that one compound, **7a**, was selective for inhibition of *M. tuberculosis* DHFR and did not inhibit human DHFR or yeast DHFR significantly in the assay. Other compounds were inactive or less active. Modelling the structure of **7a** into the dihydrofolate- and glycerol-binding pockets of *M. tuberculosis* DHFR rationalised the inhibition data, validating the original design of selective inhibitors and explaining the negative effect of halogenation of the 5-phenyl ring on biological activity. These modelling studies also indicated which of two low-energy conformations was required for binding and that there is a requirement for anticlockwise twist of the 5-phenyl ring relative to the pyrimidine. 5-Phenyl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine **7a** is shown here to be an interesting lead compound for further evaluation and further refinement of design for optimisation of potency and selectivity of inhibition of *M. tuberculosis* DHFR and, hence, new approaches to treatment of this widespread disease.

6. Experimental Section

6.1. General

NMR spectra were recorded on JEOL/Varian GX270 and EX400 spectrometers of samples in $CDCl_3$, unless otherwise stated. Mass spectra were obtained using a VG7070E spectrometer. IR spectra were measured as thin films or as KBr discs on a Perkin-Elmer RXI FT-IR spectrometer. Optical rotations were measured in a 10 cm cell on an Optical Activity Ltd. polarimeter; c is expressed in g per 100 mL. The stationary phase for chromatography was silica gel. All reactions were carried out under N_2 at ambient temperature, unless otherwise stated. Solvents were evaporated under reduced pressure. Melting points were determined by using a Reichert-Jung Thermo Galen instrument and are uncorrected.

6.2. 1-(4-Chlorophenyl)-1-cyano-2-methoxybut-1-ene (64) and 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine (pyrimethamine) (3)

Compound **62**/**63** was treated with CH_2N_2, as for the synthesis of **24a**, to give **64** (88%) as a pale yellow oil: IR ν_{max} 2204, 1606 cm^{-1}; NMR 1.32 (3 H, t, *J* = 7.6 Hz, CMe), 2.80 (2 H, q, *J* = 7.6 Hz, CH_2), 3.88 (3 H, s, OMe), 7.31 (2 H, d, *J* = 8.6 Hz, Ph 3,5-H_2), 7.61 (2 H, d, *J* = 8.6 Hz, Ph 2,6-H_2). Compound **64** was treated with guanidine, as for the synthesis of **25a**, to give **3** (50%) as a white solid: mp 233-235ºC (lit.[16] mp 233-234ºC); NMR δ_H 0.97 (3 H, t, *J* = 7.4 Hz, Me), 2.09 (2 H, q, *J* = 7.4 Hz, CH_2), 5.64 (2 H, br, NH_2), 5.92 (2 H, br, NH_2), 7.22 (2 H, d, *J* = 8.2 Hz, Ph 3,5-H_2), 7.49 (2 H, d, *J* = 8.2 Hz, Ph 2,6-H_2); MS *m/z* 251.0884 (M + H) ($C_{12}H_{14}{}^{37}ClN_4$ requires 251.0877), 249.0909 (M + H) ($C_{12}H_{14}{}^{35}ClN_4$ requires 311.0910).

6.3. 5-Phenyl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7a). Method A

Compound **26a** (150 mg, 0.4 mmol) was treated with Na (84 mg, 3.6 mmol) in liquid NH_3 (10 mL) and THF (5 mL) at -33°C for 20 min. Saturated aq. NH_4Cl (2 mL) was added and the mixture was allowed to warm to 20°C. $CHCl_3$ (14 mL) and MeOH (7 mL) were added and the mixture was filtered. Evaporation and chromatography ($CHCl_3$ / MeOH 7:3) gave **7a** (90 mg, 78%) as a white solid: mp 90-91°C; NMR (D_2O) δ_H 1.29-1.36 (1H, m, 2-H), 1.49-1.55 (1H, m, 2-H), 2.07 (1H, ddd, *J* = 13.0, 10.2, 6.2 Hz, 1-H), 2.21 (1H, ddd, *J* = 13.0, 10.5, 5.3 Hz, 1-H), 3.17-3.21 (1H, m, 3-H), 3.22-3.25 (2H, m, 5-H_2), 3.37 (1H, dt, *J* = 8.5, 6.1 Hz, 4-H), 7.03 (1H, d, *J* = 7.5 Hz, Ph 2-H), 7.04 (1H, d, *J* = 7.5 Hz, Ph 6-H), 7.23 (1H, t, *J* = 7.5 Hz, Ph 4-H), 7.29 (2H, t, *J* = 7.5 Hz, Ph 3,5-H_2); NMR (D_2O) δ_C 30.24 (CH_2), 31.10 (CH_2), 62.25 (5-

C), 71.35 (CH), 74.11 (CH), 109.19 (Pyr 5-C), 128.08 (Ph CH), 129.19 (2 × Ph CH), 130.54 (2 × Ph CH), 133.88 (Ph 1-C), 161.15 (Pyr 2-C), 162.92 (Pyr 4-C), 165.94 (Pyr 6-C); MS *m/z* 305.1616 (M + H) ($C_{15}H_{21}N_4O_3$ requires 305.1613), 327 (M + Na), 243 (M - $C_2H_5O_2$), 213 (M – $C_3H_7O_3$).

6.4. 5-Phenyl-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7a). Method B

Compound **30a** was treated with aq. CF_3CO_2H, as for the synthesis of **25a** (reaction time 6 h), to give **7a** (85%) as a white solid, with data as above.

6.5. 5-(4-Chlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7b). Method A

Compound **26b** (60 mg, 0.14 mmol) was stirred with anhydrous $FeCl_3$ (68 mg, 0.42 mmol) in dry CH_2Cl_2 (5 mL) under N_2 for 80 min. Water (2 mL) was added. Evaporation and chromatography ($CHCl_3$ / MeOH 7:3) gave **7b** (30 mg, 63%) as a white solid: $[\alpha]^{20}_D$ = -1.0° (c 1.1, MeOH); mp 250-251°C; NMR (D_2O) δ_H 1.44 (1 H, m, 2'-H), 1.65 (1 H, m, 2'-H), 2.22 (1 H, ddd, *J* = 13.6, 10.4, 6.0 Hz, 1'-H), 2.36 (1 H, ddd, *J* = 13.6, 10.0, 5.2 Hz, 1'-H), 3.31 (1 H, m, 3'-H), 3.35-3.38 (2 H, m, 5'-H_2), 3.48-3.53 (1 H, m, 4'-H), 7.18 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.44 (2 H, d, *J* = 8.5 Hz, Ar 3,5-H_2); NMR (CD_3OD) δ_C 30.05, 31.36, 62.70, 71.97, 74.14, 107.03, 129.18, 132.23, 133.13, 133.71, 161.73, 162.08, 163.06; MS *m/z* 341.1185 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_3$ requires 341.1194), 339.1225 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_3$ requires 339.1223), 308/306 (M - CH_3OH), 249/247 (M – $C_3H_7O_3$).

6.6. 5-(4-Chlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7b). Method B

Compound **30b** was treated with aq. CF_3CO_2H, as for the synthesis of **7a**, to give **7b** (91%) as a white solid, with data as above.

6.7. 5-(4-Bromophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7c). Method A

Compound **26c** was treated with $FeCl_3$, as for the synthesis of **7b**, to give **7c** (85%) as a white solid: $[\alpha]^{20}_D$ = -4.2° (c 0.24, MeOH); mp 198-200°C; NMR (D_2O) δ_H 1.47 (1 H, m, 2'-H), 1.82 (1 H, m, 2'-H), 2.19 (1 H, m, 1'-H), 2.33 (1 H, m, 1'-H), 3.29 (1 H, m, 3'-H), 3.32-3.36 (2 H, m, 5'-H_2), 3.48 (1 H, m, 4'-H), 7.08 (2 H, d, *J* = 8.0 Hz, Ar 2,6-H_2), 7.55 (2 H, d, *J* = 8.0

Hz, Ar 3,5-H_2); MS *m/z* 385.0683 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O_3$ requires 385.0698), 383.0714 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O_3$ requires 383.0718).

6.8. 5-(4-Bromophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7c). Method B

Compound **30c** was treated with aq. CF_3CO_2H, as for the synthesis of **7a**, to give **7c** (87%) as a pale yellow solid, with data as above.

6.9. 5-(3,4-Dichlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7d). Method A

Compound **26d** was treated with $FeCl_3$, as for the synthesis of **7b**, to give **7d** (77%) as a white solid: $[\alpha]^{20}_D$ = -1.4° (c 2.2, MeOH); mp 180-181°C; NMR ($(CD_3)_2SO$) 1.43 (1 H, m, 2'-H), 1.63 (1 H, m, 2'-H), 2.08 (1 H, ddd, *J* = 13.6, 10.4, 5.5 Hz, 1'-H), 2.34 (1 H, ddd, *J* = 13.6, 10.4, 5.5 Hz, 1'-H), 3.17 (1 H, m, 3'-H), 3.28-3.31 (2 H, m, 5'-H_2), 3.48 (1 H, m, 4'-H), 5.79 (2 H, br, NH_2), 5.97 (2 H, br, NH_2), 7.16 (1 H, dd, *J* = 8.2, 1.8 Hz, Ar 6-H), 7.42 (1 H, d, *J* = 1.8 Hz, Ar 2-H), 7.66 (1 H, d, *J* = 8.2 Hz, Ar 5-H); NMR ($(CD_3)_2SO$) δ_C 31.26, 32.09, 63.94, 71.95, 75.19, 105.22, 131.38, 131.81, 133.16, 133.74, 133.97, 137.51, 162.42, 166.19; MS *m/z* 377.0794 (M + H) ($C_{15}H_{19}{}^{37}Cl_2N_4O_3$ requires 377.0775), 375.0814 (M + H) ($C_{15}H_{19}{}^{37}Cl\,{}^{35}ClN_4O_3$ requires 375.0804), 373.0836 (M + H) ($C_{15}H_{19}{}^{35}Cl_2N_4O_3$ requires 373.0834), 345/343/341 (M - CH_3O).

6.10. 5-(3,4-Dichlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7d). Method B

Compound **30d** was treated with aq. CF_3CO_2H, as for the synthesis of **7a**, to give **7d** (77%) as a white solid, with data as above.

6.11. 5-Phenyl-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8a)

Compound **43a** (200 mg, 0.5 mmol) was stirred in MeOH (20 mL) with Pd/C (5%, 154 mg) and $CHCl_3$ (100 μL) under H_2 for 2 h. Filtration (Celite®), evaporation and chromatography ($CHCl_3$ / MeOH 7:3) gave **8a** (150 mg, 93%) as a white solid: mp >350 °C; $[\alpha]^{20}_D$ = +10.9° (c 0.5, H_2O); NMR (D_2O) δ_H 1.50-1.64 (2 H, m, 2'-H_2), 2.28 (1 H, ddd, *J* = 13.9, 10.1, 6.3 Hz, 1'-H), 2.40 (1 H, ddd, *J* = 13.9, 10.1, 6.3 Hz, 1'-H), 3.31-3.45 (4 H, m, 3',4',5'-H_4), 7.25 (2 H, d, *J* = 7.0 Hz, Ph 2,6-H_2), 7.42 (1 H, t, *J* = 7.0 Hz, Ph 4-H), 7.47 (2 H, t, *J* = 7.0 Hz, Ph 3,5-

H_2); NMR (D_2O) δ_C 29.75, 31.57, 62.63, 73.61, 73.64, 109.24, 128.37, 129.37, 130.56, 133.41, 160.29, 161.05, 161.81; MS *m/z* 305.1618 (M + H) ($C_{15}H_{21}N_4O_3$ requires 305.1613).

6.12. 5-(4-Chlorophenyl)-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8b)

Compound **43b** was treated with $FeCl_3$, as for the synthesis of **7b**, to give **8b** (77%) as a white solid: mp >350 °C; $[\alpha]^{20}_D$ = +6.0° (c 0.67, H_2O); NMR (CD_3OD) δ_H 1.73-1.77 (2 H, m, 2'-H_2), 2.41 (1 H, dt, *J* = 14.4, 6.7 Hz, 1'-H), 2.54 (1 H, dt, *J* = 14.4, 6.7 Hz, 1'-H), 3.44-3.61 (4 H, m, 3',4',5'-H_4), 7.31 (2 H, d, *J* = 8.4 Hz, Ar 2,6-H_2), 7.53 (2 H, d, *J* = 8.4 Hz, Ar 3,5-H_2); NMR (CD_3OD) δ_C 27.08, 31.39, 62.91, 70.52, 73.58, 108.29, 129.60, 132.04, 137.45, 137.50, 156.86, 157.38, 157.80; MS *m/z* 341.1180 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_3$ requires 341.1194), 339.1237 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_3$ requires 339.1223).

6.13. 5-(4-Bromophenyl)-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8c)

Compound **43c** was treated with $FeCl_3$, as for the synthesis of **7b**, to give **8c** (77%) as a white solid: mp >350 °C; $[\alpha]^{20}_D$ = +12.5° (c 0.24, MeOH); IR ν_{max} 3649, 3468, 3418, 1618 cm^{-1}; NMR (D_2O) δ_H 1.60-1.72 (2 H, m, 2'-H_2), 2.30-2.54 (2 H, m, 1'-H_2), 3.42-3.56 (4 H, m, 3',4',5'-H_4), 7.25 (2 H, d, *J* = 8.7 Hz, Ar 2,6-H_2), 7.72 (2 H, d, *J* = 8.7 Hz, Ar 3,5-H_2); MS *m/z* 385.0707 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O_3$ requires 385.0698), 383.0717 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O_3$ requires 383.0718).

6.14. 1-Cyano-7-hydroxy-2-methoxy-1-phenylhept-1-ene (47a) and 6-(5-hydroxypentyl)-5-phenylpyrimidine-2,4-diamine (9a)

Compound **45a**/**46a** was treated with CH_2N_2, as for the synthesis of **24a** (followed by chromatography (EtOAc / hexane 2:1)), to give **47a** (78%) as a pale yellow oil: IR ν_{max} 3439, 2204 cm^{-1}; MS *m/z* 246.1492 (M + H) ($C_{15}H_{20}NO_2$ requires 246.1494). Compound **47a** was treated with guanidine, as for the synthesis of **25a** (chromatographic eluant CH_2Cl_2 / MeOH (4:1)), to give **9a** (36%) as a white solid: mp 214-216ºC; IR ν_{max} 3420, 3331, 3177, 1619 cm^{-1}; NMR δ_H 1.27 (2 H, qn, *J* = 7.2 Hz, 3'-H_2), 1.45 (2 H, qn, *J* = 7.2 Hz, 4'-H_2), 1.55 (2 H, qn, *J* = 7.2 Hz, 2'-H_2), 2.28 (2 H, t, *J* = 7.2 Hz, 1'-H_2), 3.56 (2 H, t, *J* = 6.4 Hz, 5'-H_2), 4.59 (2 H, br, NH_2), 4.98 (2 H, br, NH_2), 7.21 (2 H, d, *J* = 7.2 Hz, Ph 2,6-H_2), 7.37 (1 H, t, *J* = 7.2 Hz, Ph 4-H), 7.44 (2 H, t, *J* = 7.2 Hz, Ph 3,5-H_2); NMR (CD_3OD) δ_C 25.38, 28.49, 31.81, 33.79, 61.33, 108.29, 127.67, 128.95, 130.51, 134.76, 161.32, 162.98, 165.09; MS *m/z* 273.1704 (M + H) ($C_{15}H_{20}N_4O$ requires 273.1715), 213 (M – C_3H_7O), 200 (M – C_4H_8O).

6.15. 1-(4-Chlorophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (47b) and 5-(4-chlorophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9b)

Compound **45b**/**46b** was treated with CH_2N_2, as for the synthesis of **24a** (followed by chromatography (EtOAc / hexane 3:1)), to give **47b** (62%) as a pale yellow oil: NMR δ_H 1.52-1.80 (6 H, m, 4,5,6-H_6), 2.77 (2 H, t, *J* = 7.8 Hz, 3-H_2), 3.68 (2 H, t, *J* = 6.2 Hz, 7-H_2), 3.85 (3 H, s, Me), 7.29 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.54 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); MS *m/z* 282.1077 (M + H) ($C_{15}H_{19}{}^{37}ClNO_2$ requires 280.1074), 280.1102 (M + H) ($C_{15}H_{19}{}^{35}ClNO_2$ requires 280.1104), 264/262 (M – OH). Compound **47b** was treated with guanidine, as for the synthesis of **9a**, to give **9b** (59%) as a white solid: mp 165-166ºC; IR ν_{max} 3407, 3329, 3174, 1631 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.11 (2 H, qn, *J* = 7.4 Hz, 3'-H_2), 1.26 (2 H, qn, *J* = 7.4 Hz, 4'-H_2), 1.42 (2 H, qn, *J* = 7.4 Hz, 2'-H_2), 2.07 (2 H, t, *J* = 7.4 Hz, 1'-H_2), 3.29 (2 H, t, *J* = 7.4 Hz, 5'-H_2), 4.29 (1 H, br, OH), 5.64 (2 H, br, NH_2), 5.94 (2 H, br, NH_2) 7.18 (2 H, d, *J* = 8.2 Hz, Ar 2,6-H_2), 7.47 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2); NMR ($(CD_3)_2SO$) δ_C 25.88, 28.58, 32.69, 34.63, 61.00, 106.16, 129.34, 132.25, 134.76, 133.10, 135.47, 162.44, 162.47, 165.87; MS *m/z* 309.1310 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_2$ requires 309.1296), 307.1335 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_2$ requires 307.1325), 236/234 (M - C_4H_8O).

6.16. 1-(4-Bromophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (47c) and 5-(4-bromophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9c)

Compound **45c**/**46c** was treated with CH_2N_2, as for the synthesis of **47b**, to give **47c** (32%) as a pale yellow oil: NMR δ_H 1.53-1.75 (6 H, m, 4,5,6-H_6), 2.76 (2 H, t, *J* = 7.0 Hz, 3-H_2), 3.69 (2 H, t, *J* = 7.0 Hz, 7-H_2), 3.85 (3 H, s, Me), 7.44 (2 H, d, *J* = 8.8 Hz, Ar 2,6-H_2), 7.48 (2 H, d, *J* = 8.8 Hz, Ar 3,5-H_2); MS *m/z* 326.0583 (M + H) ($C_{15}H_{19}{}^{81}BrNO_2$ requires 326.0578), 324.0596 (M + H) ($C_{15}H_{19}{}^{79}BrNO_2$ requires 324.0599). Compound **47c** was treated with guanidine, as for the synthesis of **9a**, to give **9c** (43%) as a white solid: mp 177-178ºC; IR ν_{max} 3550, 3468, 3414, 1617 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.11 (2 H, qn, *J* = 7.4 Hz, 3'-H_2), 1.25 (2 H, qn, *J* = 7.4 Hz, 4'-H_2), 1.42 (2 H, qn, *J* = 7.4 Hz, 2'-H_2), 2.07 (2 H, t, *J* = 7.4 Hz, 1'-H_2), 3.27 (2 H, t, *J* = 6.4 Hz, 5'-H_2), 4.30 (1 H, br, OH), 5.74 (2 H, br, NH_2), 6.00 (2 H, br, NH_2), 7.11 (2 H, d, *J* = 8.4 Hz, Ar 2,6-H_2), 7.57 (2 H, d, *J* = 8.4 Hz, Ar 3,5-H_2); NMR (CF_3CO_2H salt) ($(CD_3)_2SO$) δ_C 25.33, 27.72, 30.33, 32.21, 60.68, 108.00, 115.78 (q, *J* = 289.1 Hz), 122.75, 130.74, 132.74, 133.24, 153.43, 155.31, 158 (q, *J* = 37.6 Hz), 164.30; MS *m/z* 353.0807 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O$ requires 353.0800), 351.0816 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O$ requires 351.0820).

6.17. 1-Cyano-1-(3,4-dichlorophenyl)-7-hydroxy-2-methoxyhept-1-ene (47d) and 5-(3,4-dichlorophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9d)

Compound **45c**/**46c** was treated with CH_2N_2, as for the synthesis of **47b**, to give **47d** (68%) as a pale yellow oil: NMR δ_H 1.41-1.50 (4 H, m, 5,6-H_4), 1.62 (2 H, qn, *J* = 7.6 Hz, 4-H_2), 2.75 (2 H, t, *J* = 7.6 Hz, 3-H_2), 3.41 (2 H, t, *J* = 6.0 Hz, 7-H_2), 3.95 (3 H, s, Me), 7.50 (1 H, dd, *J* = 8.6, 2.0 Hz, Ar 6-H), 7.63 (1 H, d, *J* = 8.6 Hz, Ar 5-H), 7.75 (1 H, d, *J* = 2.0 Hz, Ar 2-H); MS *m/z* 318.0686 (M + H) ($C_{15}H_{18}{}^{37}Cl_2NO_2$ requires 318.0655), 316.0689 (M + H) ($C_{15}H_{18}{}^{37}Cl^{35}ClNO_2$ requires 316.0685), 314.0715 (M + H) ($C_{15}H_{18}{}^{35}Cl_2NO_2$ requires 314.0714), 291/289/287 (M – CN). Compound **47d** was treated with guanidine, as for the synthesis of **9a**, to give **9d** (43%) as a white solid: mp 94-95ºC; IR ν_{max} 3499, 3419, 3333, 1622 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.12 (2 H, qn, *J* = 7.4 Hz, 3'-H_2), 1.25 (2 H, qn, *J* = 7.4 Hz, 4'-H_2), 1.42 (2 H, qn, *J* = 7.4 Hz, 2'-H_2), 2.07 (2 H, t, *J* = 7.4 Hz, 1'-H_2), 3.27 (2 H, q, *J* = 5.6 Hz, 5'-H_2), 4.28 (1 H, t, *J* = 5.6 Hz, OH), 5.72 (2 H, br, NH_2), 5.90 (2 H, br, NH_2), 7.11 (1 H, dd, *J* = 8.2, 2.2 Hz, Ar 6-H), 7.36 (1 H, d, *J* = 2.2 Hz, Ar 2-H), 7.62 (1 H, d, *J* = 8.2 Hz, Ar 5-H); NMR ($(CD_3)_2SO$) δ_C 25.87, 28.50, 32.70, 34.64, 61.02, 105.23, 130.21, 131.37, 131.74, 131.81, 133.19, 137.66, 162.36, 162.69, 165.96; MS *m/z* 345.0885 (M + H) ($C_{15}H_{19}{}^{37}Cl_2N_4O$ requires 345.0876), 343.0901 (M + H) ($C_{15}H_{19}{}^{37}Cl^{35}ClN_4O$ requires 343.0906), 341.0927 (M + H) ($C_{15}H_{19}{}^{35}Cl_2N_4O$ requires 341.0935), 285/283/281 (M - C_3H_7O), 272/270/268 (M – C_4H_8O).

6.18. 5-Phenyl-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10a)

Compound **52a** was treated with aq. CF_3CO_2H, as for the synthesis of **26a** (reaction time 2 h), to give **10a** (73%) as a highly hygroscopic white solid: $[\alpha]^{20}_D$ = -0.38° (c 4, MeOH); NMR (CD_3CN) δ_H 3.45 (1 H, dd, *J* = 11.6, 4.9 Hz, 3'-H), 3.48 (1 H, dd, *J* = 11.6, 3.9 Hz, 3'-H), 3.72 (1 H, m, 2'-H), 4.46 (1 H, d, *J* = 6.2 Hz, 1'-H), 4.73 (2 H, br, NH_2), 5.82 (1 H, br, NH), 6.98 (1 H, br, NH), 7.31 (2 H, dd, *J* = 7.4, 2.0 Hz, Ph 2,6-H_2), 7.45-7.61 (3 H, m, Ph 3,4,5-H_3); MS *m/z* 299 (M + Na), 277.1308 (M + H) ($C_{13}H_{17}N_4O_3$ requires 277.1300).

6.19. 5-(4-Chlorophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10b)

Compound **52b** was treated with aq. CF_3CO_2H, as for the synthesis of **26a**, to give **10b** (90%) as a pale yellow solid: mp 196-197 ºC; $[\alpha]^{20}_D$ = -41° (c 0.4, MeOH); IR ν_{max} 3550, 3475,

3413, 1617 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 3.58 (1 H, dd, *J* = 11.3, 4.7 Hz, 3'-H, 3.63 (1 H, dd, *J* = 11.3, 3.5 Hz, 3'-H), 3.89-3.92 (1 H, m, 2'-H), 4.54 (1H, d, *J* = 7.0 Hz, 1'-H), 5.54 (5 H, br) and, 6.62 (1 H, br) (2 × NH_2 + 2 × OH), 7.40 (2 H, d, *J* = 7.4 Hz, Ar 2,6-H_2), 7.47 (2 H, d, *J* = 7.4 Hz, Ar 3,5-H_2),7.79 (1 H, br, OH); NMR ($(CD_3)_2SO$) δ_C 62.60, 69.31, 72.47, 108.42, 129.88, 132.39, 133.53, 134.72, 161.21, 161.53, 161.87; MS *m/z* 313.0877 (M + H) ($C_{13}H_{16}{}^{37}ClN_4O_3$ requires 313.0881), 311.0905 (M + H) ($C_{13}H_{16}{}^{35}ClN_4O_3$ requires 311.0910).

6.20. 5-(4-Bromophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10c)

Compound **52c** was treated with aq. CF_3CO_2H, as for the synthesis of **26a** (reaction time 4 h), to give **10c** (95%) as a highly hygroscopic pale yellow solid: $[\alpha]^{20}{}_D$ = -15° (c 0.9, MeOH); IR ν_{max} 3550, 3478, 3414, 1618 cm^{-1}; NMR (CD_3CN) δ_H 3.51 (1 H, dd, *J* = 12.3, 4.9 Hz, 3'-H), 3.55 (1 H, dd, *J* = 12.3, 4.5 Hz, 3'-H), 3.74 (1 H, m, 2'-H), 4.49 (1 H, d, *J* = 6.2 Hz, 1'-H), 5.94 (2 H, br, NH_2), 7.03 (2 H, br, NH_2), 7.29 (2 H, d, *J* = 8.0 Hz, Ar 2,6-H_2), 7.71 (2 H, d, *J* = 8.0 Hz, Ar 3,5-H_2); NMR (CD_3CN) δ_C 62.47, 68.98, 72.30, 108.83, 123.37, 129.70, 132.92, 132.98, 133.81, 156.05, 162.06, 164.91; MS *m/z* 379/377 (M + Na), 357.0396 (M + H) ($C_{13}H_{16}{}^{81}BrN_4O_3$ requires 357.0385), 355.0412 (M + H) ($C_{13}H_{16}{}^{79}BrN_4O_3$ requires 355.0405).

6.21. 5-(3,4-Dichlorophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10d)

Compound **52d** was treated with aq. CF_3CO_2H, as for the synthesis of **10c**, to give **10d** (87%) as a pale yellow solid: mp 120-121 ºC; $[\alpha]^{20}{}_D$ = -3.0° (c 4.7, MeOH); IR ν_{max} 3549, 3476, 3415, 1618 cm^{-1}; NMR (CD_3CN) δ_H 3.41 (1 H, d, *J* = 13.1 Hz, 3'-H), 3.45 (1 H, d, *J* = 13.1 Hz, 3'-H), 3.63-3.68 (1 H, m, 2'-H), 4.35 (1 H, d, *J* = 4.7 Hz, 1'-H), 5.25 (2 H, br, NH_2), 5.67 (2 H, br, NH_2), 7.20 (1 H, dd, *J* = 8.0, 1.9 Hz, Ar 6-H), 7.46 (1 H, d, *J* = 1.9 Hz, Ar 2-H), 7.60 (1 H, d, *J* = 8.0 Hz, Ar 5-H); NMR ($(CD_3)_2SO$) δ_C 63.67, 69.38, 74.29, 106.16, 130.30, 131.08, 131.44, 131.63, 132.68, 134.09, 162.01, 162.81, 164.00; MS *m/z* 371/369/367 (M + Na), 349.0469 (M + H) ($C_{13}H_{15}{}^{37}Cl_2N_4O_3$ requires 349.0462), 347.0501 (M + H) ($C_{13}H_{15}{}^{37}Cl^{35}ClN_4O_3$ requires 347.0491), 345.0521 (M + H) ($C_{13}H_{15}{}^{35}Cl_2N_4O_3$ requires 345.0521).

6.22. 1-Cyano-1,4-diphenyl-2-methoxybut-1-ene (56) and 5-phenyl-6-(2-phenylethyl)-pyrimidine-2,4-diamine (11)

Compound **54**/**55** was treated with CH_2N_2, as for the synthesis of **24a**, to give **56** (95%) as a pale yellow oil: IR ν_{max} 2204 cm^{-1}; MS *m/z* 264.1390 (M + H) ($C_{18}H_{18}NO$ requires 264.1388), 236 (M – HCN), 91 (Bn). Compound **56** was treated with guanidine, as for the synthesis of **25a** (chromatographic eluant CH_2Cl_2 / MeOH (8:1)), to give **11** (32%) as a pale yellow solid: mp 116-118ºC; NMR δ_H 2.54 (2 H, t, *J* = 8.0 Hz, CH_2), 2.83 (2 H, t, *J* = 8.0 Hz, CH_2), 4.62 (2 H, br, NH_2), 4.99 (2 H, br, NH_2), 6.94 (2 H, d, *J* = 6.8 Hz, Ph 2,6-H_2), 7.05 (2 H, d, *J* = 6.5 Hz, Ph' 2,6-H_2), 7.14 (1H, t, *J* = 6.8 Hz, Ph 4-H), 7.17 (2 H, t, *J* = 6.8 Hz, Ph 3,5-H_2) 7.35 (2 H, t, *J* = 6.5 Hz, Ph' 4-H), 7.39 (2 H, t, *J* = 6.5 Hz, Ph' 3,5-H_2); MS *m/z* 291.1616 (M + H) ($C_{18}H_{19}N_4$ requires 291.1609), 199 (M – Bn).

6.23. 1-Cyano-2-methoxy-1-phenylprop-1-ene (60) and 6-methyl-5-phenylpyrimidine-2,4-diamine (12)

Compound **58**/**59** was treated with CH_2N_2, as for the synthesis of **24a**, to give **60** (87%) as a pale yellow oil: IR ν_{max} 2204, 1606 cm^{-1}; NMR δ_H 2.45 (3 H, s, CMe), 3.85 (3 H, s, OMe), 7.26 (1 H, t, *J* = 7.0 Hz, Ph 4-H), 7.30 (2 H, t, *J* = 7.0 Hz, Ph 3,5-H_2), 7.61 (2 H, t, *J* = 7.0 Hz, Ph 2,6-H_2); MS *m/z* 174.0921 (M + H) ($C_{11}H_{12}NO$ requires 174.0918). Compound **60** was treated with guanidine, as for the synthesis of **25a** (chromatographic eluant CH_2Cl_2 / MeOH (4:1)), to give **12** (38%) as a pale yellow solid: mp 250-251ºC (lit.[19] mp 249-251ºC); IR ν_{max} 3395, 3323 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.85 (3 H, s, Me), 5.62 (2 H, br, NH_2), 6.00 (2 H, br, NH_2), 7.20 (2 H, d, *J* = 7.3 Hz, Ph 2,6-H_2), 7.33 (1 H, t, *J* = 7.3 Hz, Ph 4-H), 7.43 (2 H, t, *J* = 7.3 Hz, Ph 3,5-H_2); MS *m/z* 201.1145 (M + H) ($C_{11}H_{13}N_4$ requires 201.1140), 123 (M – C_6H_5), 109 (M – C_7H_7).

6.24. 2,3-O-Isopropylidene-L-erythrose (18)

L-Arabinose **17** (10.0 g, 67 mmol), TsOH.H_2O (150 mg, 0.79 mmol) and 2,2-dimethoxy-propane (23.0 g, 221 mol) were stirred in dry DMF (130 mL) under N_2 for 90 min. The mixture was neutralised with Na_2CO_3. The evaporation residue was added to water (120 mL) and hexane (60 mL). $NaIO_4$ (35.5 g, 0.17 mol) was added to the aq. layer and the mixture was stirred for 2 h. Na_2CO_3 was added and the slurry was stirred for 1 h. The mixture was extracted with EtOAc. Evaporation and chromatography (Et_2O / hexane 2:1) gave **18** (5.8 g,

54%) as a colourless oil (lit.[45] oil): NMR δ_H 1.31 (3 H, s, Me), 1.46 (3 H, s, Me), 3.89 (1 H, d, *J* = 2.5 Hz, OH), 4.01 (1 H, d, *J* = 10.5 Hz, 4-H), 4.05 (1 H, dd, *J* =10.5, 3.5 Hz, 4-H), 4.55 (1 H, d, *J* = 6.0 Hz, 2-H), 4.82 (1 H, dd, *J* = 6.0, 3.5 Hz, 3-H), 5.39 (1 H, d, *J* = 2.5 Hz, 1-H); MS *m/z* 181 (M + Na), 159.0650 (M - H) ($C_7H_{11}O_4$ requires 159.0657).

6.25. Ethyl (*Z,4S,5R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (19*Z*) and ethyl (*E,4S,5R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (19*E*)

Ethyl triphenylphosphoranylidineacetate (9.3 g, 27 mmol) was stirred with **18** (2.9 g, 18 mmol) in CH_2Cl_2 (130 mL) for 16 h. The evaporation residue was extracted with Et_2O. Evaporation and chromatography (Et_2O / hexane 1:1) gave **19*Z*** (2.2 g, 54%) as a colourless oil (lit.[46] oil): NMR δ_H 1.29 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.40 (3 H, s, 2-Me), 1.53 (3 H, s, 2-Me), 2.44 (1 H, dd, *J* = 7.4, 5.5 Hz, OH), 3.45 (1 H, m, CH*H*OH), 3.59 (1 H, m, C*H*HOH), 4.16 (2 H, q, *J* = 7.4 Hz, CH_2CH_3), 4.53-4.57 (1 H, m, 4-H), 5.58 (1 H, dt, *J* = 7.1, 1.7 Hz, 5-H), 5.91 (1 H, dd, *J* = 11.7, 1.7 Hz, $CHCO_2$), 6.36 (1 H, dd, *J* = 11.7, 7.1 Hz, $CH{=}CCO_2$). Further elution gave **19*E*** (600 mg, 15%) as a colourless oil (lit.[46] oil): NMR δ_H 1.29 (3 H, t, *J* = 7.2 Hz, CH_2CH_3), 1.40 (3 H, s, 2-Me), 1.52 (3 H, s, 2-Me), 2.41 (1 H, t, *J* = 5.9 Hz, OH), 3.55 (2 H, t, *J* = 5.9 Hz, CH_2OH), 4.18 (2 H, q, *J* = 7.2 Hz, CH_2CH_3), 4.35 (1 H, m, 4-H), 4.79 (1H, dt, *J* = 5.5, 1.6 Hz, 5-H), 6.12 (1 H, dd, *J* = 15.6, 1.6 Hz, $CHCO_2$), 6.88 (1 H, dd, *J* = 15.6, 5.5 Hz, $CH{=}CCO_2$); MS *m/z* 231.1240 (M + H) ($C_{11}H_{19}O_5$ requires 231.1232), 215 (M - CH_3), 173 (M – C_3H_5O), 143 (M – $C_4H_7O_2$).

6.26. Ethyl (4*S*,5*R*)-4-hydroxymethyl-2,3-dimethyl-1,3-dioxolane-5-propanoate (20)

A mixture of **19*Z*** and **19*E*** (2.3 g, 10 mmol) was stirred in EtOH (100 mL) with Pd/C (5%, 150 mg) under H_2 for 3 h. Filtration (Celite®) and evaporation gave **20** (2.3 g, 99%) as a pale yellow oil (lit.[46] oil): NMR δ_H 1.26 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.33 (3 H, s, 2-Me), 1.42 (3 H, s, 2-Me), 1.82 (2 H, m, $CH_2CH_2CO_2$), 2.39 (1 H, br, OH), 2.40 (1 H, dt, *J* = 16.4, 7.8 Hz, $CHCO_2$), 2.53 (1 H, dt, *J* = 16.4, 7.4 Hz, $CHCO_2$), 3.65 (2 H, d, *J* = 5.1 Hz, CH_2OH), 4.09-4.20 (4 H, m, 4-H + 5-H + CH_2CH_3); MS *m/z* 233.1396 (M + H) ($C_{11}H_{19}O_5$ requires 233.1388), 217 (M - CH_3).

6.27. Ethyl (4*S*,5*R*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate (21)

$LiN(SiMe_3)_2$ (1.0 M in THF, 10 mL, 10 mmol) was stirred with **20** (2.3 g, 10 mmol) and BnBr (3.4 g, 20 mmol) in dry DMF (5 mL). After 2 h, water was added. The mixture was ex-

tracted (Et_2O). The extract was washed with water and brine and was dried. Evaporation and chromatography (Et_2O / hexane 1:4) afforded **21** (1.6 g, 48%) as a pale yellow oil: $[\alpha]^{20}_D$ = +24.8° (c 4.4, $CHCl_3$); NMR δ_H 1.24 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.33 (3 H, s, 2-Me), 1.42 (3 H, s, 2-Me), 1.72-1.86 (2 H, m, $CH_2CH_2CO_2$), 2.50 (1 H, m, $CHCO_2$), 2.48-2.54 (1 H, m, $CHCO_2$), 3.50 (2 H, m, CH_2OBn), 4.08-4.15 (3 H, m, 5-H + CH_2CH_3), 4.28 (1 H, dd, *J* = 11.9, 6.1 Hz, 4-H), 4.50 (1 H, d, *J* = 12.1 Hz, CHPh), 4.57 (1 H, d, *J* = 12.1 Hz, CHPh), 7.24-7.33 (5H, m, Ph-H_5); MS *m/z* 323.1864 (M + H) ($C_{18}H_{27}O_5$ requires 323.1858), 265 (M – C_3H_5O), 91 (Bn).

6.28. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane (22a) / (4*R*,5*S*)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (23a)

$LiN(SiMe_3)_2$ (1.0 M in THF, 9.1 mL, 9.1 mmol) was added to phenylacetonitrile (1.1 g, 9.4 mmol) in dry Et_2O (10 mL) under N_2 at -78°C. After 10 min, **21** (2.9 g, 9.0 mmol) was added. The mixture was allowed to warm to 20°C and was stirred for 72 h. Water was added. The solution was washed twice (Et_2O) before being acidified to pH 6 with aq. citric acid (1 M) in the presence of EtOAc. The EtOAc phase was separated and washed with water. Drying, evaporation and chromatography (EtOAc / hexane, 2:1) gave **22a**/**23a** (1.5 g, 21%) as a yellow oil: IR ν_{max} 2207, 1728 cm^{-1}; NMR δ_H 1.26 (3 H, s, 2-Me), 1.34 (3 H, s, 2-Me), 1.66-1.80 (2 H, m, CH_2CH_2CO), 2.63 (1 H, m, CHCO), 2.75-2.80 (1 H, m, CHCO), 3.45 (2 H, d, *J* = 6.0 Hz, CH_2OBn), 3.98 (1 H, m, 4-H), 4.20 (1 H, q, *J* = 6.0 Hz, 5-H), 4.45 (1 H, d, *J* = 11.5 Hz, CHPh), 4.52 (1 H, d, *J* = 11.5 Hz, CHPh), 7.21-7.41 (10 H, m, 2 × Ph-H_5), 8.98 (1 H, s, OH); MS *m/z* 394.2016 (M + H) ($C_{24}H_{28}NO_4$ requires 394.2018), 91 (Bn).

6.29. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22b) / (4*R*,5*S*)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(4-chlorophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23b)

4-Chlorophenylacetonitrile and **21** were condensed as for the synthesis of **22a**/**23a** (chromatographic eluant EtOAc / hexane (1:1)), to give **22b**/**23b** (26%) as a yellow oil: NMR δ_H 1.25 (2.7 H, s, Me), 1.34 (2.7 H, s, Me), 1.40 (0.3 H, s, Me), 1.51 (0.3 H, s, Me), 1.67-1.88 (2 H, m, CH_2CH_2CO), 1.98-2.08 (0.2 H, m, CH_2CO), 2.60-2.80 (1.8 H, m, CH_2CO), 3.45 (1 H, dd, *J* = 12.1, 6.0 Hz, CHOBn), 3.47 (1 H, dd, *J* = 12.1, 6.0 Hz, CHOBn), 4.00 (0.9 H, ddd, *J* = 8.6, 6.0, 2.3 Hz, 4-H), 4.22 (0.9 H, q, *J* = 6.0 Hz, 5-H), 4.29 (0.1 H, ddd, *J* = 10.14, 6.0, 3.9

Hz, 4-H), 4.39 (0.1 H, q, *J* = 6.0 Hz, 5-H), 4.45 (0.1 H, d, *J* = 11.5 Hz, CHPh), 4.49 (0.1 H, d, *J* = 11.5 Hz, CHPh), 4.51 (0.9 H, d, *J* = 11.9 Hz, CHPh), 4.56 (0.9 H, d, *J* = 11.9 Hz, CHPh), 5.52 (0.1 H, s, CHCN), 7.16 (0.2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.25-7.32 (5 H, m, Ph-H_5), 7.35 (1.8 H, d, *J* = 8.6 Hz, Ar 3,5-H_2), 7.84 (1.8 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 9.33 (0.9 H, br, OH); MS *m/z* 430.1604 (M + H) ($C_{24}H_{26}{}^{37}ClNO_4$ requires 430.1599), 428.1618 (M + H) ($C_{24}H_{27}{}^{35}ClNO_4$ requires 428.1628), 370 (M - C_2H_4NO), 91 (Bn).

6.30. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22c) / (4*R*,5*S*)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(4-bromophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23c)

4-Bromophenylacetonitrile and **21** were condensed, as for the synthesis of **22a**/**23a**, to give **22c**/**23c** (18%) as a pale yellow oil: NMR δ_H 1.27 (3 H, s, 2-Me), 1.35 (3 H, s, 2-Me), 1.70-1.82 (2H, m, C*H*$_2$CHO), 2.62-2.78 (2 H, m, CH_2C=O), 3.45-3.47 (2 H, m, CH_2OBn), 4.00 (1 H, ddd, *J* = 10.1, 6.2, 3.9 Hz, 4-H), 4.22 (1 H, q, *J* = 6.2 Hz, 5-H), 4.45 (1 H, d, *J* = 11.9 Hz, CHPh), 4.49 (1 H, d, *J* = 11.9 Hz, CHPh), 5.48 (0.35 H, s, CHCN), 7.21-7.35 (5 H, m, Ph-H_5), 7.45 (1.3 H, d, *J* = 8.8 Hz, Ar 2,6-H_2), 7.55 (1.3 H, d, *J* = 8.8 Hz, Ar 3,5-H_2), 7.59 (0.7 H, d, *J* = 8.6 Hz, Ar 3,5-H_2), 7.76 (0.7 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 9.35 (0.65 H, s, OH); MS *m/z* 474.1100 (M + H) ($C_{24}H_{27}{}^{81}BrNO_4$ requires 474.1102), 472.1094 (M + H) ($C_{24}H_{27}{}^{79}BrNO_4$ requires 472.1123), 415/413 (M - C_2H_4NO), 91 (Bn).

6.31. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(3,4-dichlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22d) / (4*R*,5*S*)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(3,4-dichlorophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23d)

3,4-Dichlorophenylacetonitrile and **21** were condensed, as for the synthesis of **22a**/**23a**, to give **22d**/**23d** (16%) as a highly hygroscopic white solid: NMR δ_H 1.41 (3 H, s, 2-Me), 1.52 (3 H, s, 2-Me), 1.73-1.87 (2 H, m, C*H*$_2$CHO), 2.71-2.84 (2 H, m, CH_2C=O), 3.47 (1 H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.49 (1 H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.98 (1 H, m, 4-H), 4.22 (1 H, q, *J* = 6.0 Hz, 5-H), 4.47 (1 H, d, *J* = 12.3 Hz, CHPh), 4.56 (1 H, d, *J* = 12.3 Hz, CHPh), 7.26-7.37 (5 H, m, Ph-H_5), 7.45 (1 H, d, *J* = 8.6 Hz, Ar 5-H), 7.50 (1 H, dd, *J* = 8.6, 2.0 Hz, Ar 6-H), 7.83 (1 H, d, *J* = 2.0 Hz, Ar 2-H), 9.61 (1 H, s, OH); MS *m/z*. 466.1178 (M + H) ($C_{24}H_{26}{}^{37}Cl_2NO_4$ requires 466.1179), 464.1193 (M + H) ($C_{24}H_{26}{}^{37}Cl^{35}ClNO_4$ requires 464.1209), 462.1217 (M + H) ($C_{24}H_{26}{}^{35}Cl_2NO_4$ requires 462.1238), 407/405/403 (M - C_2H_4NO), 91 (Bn).

6.32. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24a) and 6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (25a)

Compound **22a**/**23a** (1.5 g, 3.7 mmol) in THF (5 mL) was treated with CH_2N_2 (8.0 mmol) in Et_2O (20 mL) at 10°C for 16 h. Excess CH_2N_2 was destroyed by careful addition of AcOH (30% in THF). Evaporation gave **24a** (1.2 g, 81%) as a yellow oil: NMR δ_H 1.37 (3 H, s, 2-Me), 1.46 (3 H, s, 2-Me), 1.77-1.88 (2 H, m, C*H*$_2$CHO), 2.78-2.86 (1 H, m, CHC=C), 2.90-2.98 (1 H, m, CHC=C), 3.53 (2 H, d, *J* = 5.9 Hz, CH_2OBn), 3.78 (3 H, s, OMe), 4.22 (1 H, m, 4-H), 4.33 (1 H, m, 5-H), 4.51 (1 H, d, *J* = 12.1 Hz, CHPh), 4.59 (1 H, d, *J* = 12.1 Hz, CHPh), 7.21-7.59 (10 H, m, 2 × Ph-H$_5$); MS *m/z* 408.2166 (M + H) ($C_{25}H_{30}NO_4$ requires 408.2174), 350 (M – C_2H_3NO), 91 (Bn). NaOMe (140 g, 2.6 mmol) was stirred with guanidine.HCl (300 mg, 2.6 mmol) in MeO$(CH_2)_2$OH (10 mL) stirred for 5 min at 30°C. The filtered solution was boiled under reflux with **24a** (700 mg, 1.8 mmol) for 16 h. Evaporation and chromatography ($CHCl_3$ / MeOH 19:1) gave **25a** (400 mg, 46%) as a highly hygroscopic pale yellow solid: IR ν_{max} 3415, 1685 cm^{-1}; NMR δ_H 1.23 (3 H, s, Me), 1.24 (3 H, s, Me), 1.61-1.67 (2 H, m, C*H*$_2$CHO), 2.24 (1 H, m, Pyr-CH), 2.51 (1 H, m, Pyr-CH), 3.4 (2 H, d, *J* = 6.0 Hz, CH_2OBn), 3.95-4.00 (1 H, m, dioxolane 4-H), 4.15 (1 H, q, *J* = 6.0 Hz, dioxolane 5-H), 4.44 (1H, d, *J* = 12.3 Hz, CHPh), 4.53 (1 H, d, *J* = 12.3 Hz, CHPh), 4.68 (2 H, br, NH_2), 5.01 (2 H, br, NH_2), 7.09-7.39 (10H, m, 2 × Ph-H$_5$); MS *m/z* 435.2423 (M + H) ($C_{25}H_{31}N_4O_3$ requires 435.2396), 200 (M – $C_{14}H_{18}O_3$), 91 (Bn).

6.33. (4*R*,5*S*)-5-Benzyloxymethyl-4-(-4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24b) and 6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (25b)

Compound **22b**/**23b** was treated with CH_2N_2, as for the synthesis of **24a**, to give **24b** (97%) as a pale yellow oil: NMR δ_H 1.36 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.74 (1 H, m, C*H*CHO), 1.85 (1 H, m, C*H*CHO), 2.80 (1 H, m, CHC=C), 2.94 (1 H, m, CHC=C), 3.51 (1 H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.53 (1 H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.80 (3 H, s, OMe), 4.22 (1 H, ddd, *J* = 9.4, 6.0, 3.1 Hz, 4-H), 4.33 (1 H, q, *J* = 6.0 Hz, 5-H), 4.50 (1 H, d, *J* = 12.1 Hz, CHPh), 4.59 (1 H, d, *J* = 12.1 Hz, CHPh), 7.28 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H$_2$), 7.30-7.36 (5 H, m, Ph-H$_5$), 7.52 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H$_2$); MS *m/z* 444.1746 (M + H) ($C_{25}H_{29}{}^{37}ClNO_4$ requires 444.1755), 442.1764 (M + H) ($C_{25}H_{29}{}^{35}ClNO_4$ requires 442.1785), 428/426 (M - CH_3), 386/384 (M – C_2H_3NO), 91 (Bn). Compound **24b** was treated with

guanidine, as for the synthesis of **25a** (chromatographic eluant $CHCl_3$ / MeOH (9:1)) to give **25b** (25%) as a pale buff solid: mp 55-56°C; NMR δ_H 1.28 (6 H, s, Me_2), 1.62-1.71 (2 H, m, C*H*$_2$CHO), 2.25 (1 H, ddd, *J* = 13.5, 10.1, 5.9 Hz, Pyr-CH), 2.51 (1 H, ddd, *J* = 13.5, 10.1, 5.9 Hz, Pyr-CH), 3.43 (2 H, d, *J* = 5.9 Hz, CH_2OBn), 4.01 (1 H, ddd, *J* = 10.1, 5.9, 4.3 Hz, dioxolane 4-H), 4.21 (1 H, q, *J* = 5.9 Hz, dioxolane 5-H), 4.47 (1 H, d, *J* = 12.1 Hz, CHPh), 4.56 (1 H, d, *J* = 12.1 Hz, CHPh), 4.82 (2 H, br, NH_2), 5.23 (2 H, br, NH_2), 7.14 (1 H, d, *J* = 8.2 Hz, Ar 2-H), 7.15 (1 H, d, *J* = 8.2 Hz, Ar 6-H), 7.27-7.37 (5H, m, Ph-H_5), 7.39 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2); MS *m/z* 471.1992 (M + H) ($C_{25}H_{30}{}^{37}ClN_4O_3$ requires 471.1976), 469.2005 (M + H) ($C_{25}H_{30}{}^{35}ClN_4O_4$ requires 469.2006), 236/234 (M – $C_{14}H_{18}O_3$), 91 (Bn).

6.34. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24c) and 6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(4-bromophenyl)pyrimidine-2,4-diamine (25c)

Compound **22c**/**23c** was treated with CH_2N_2, as for the synthesis of **24a**, to give **24c** (87%) as a pale yellow oil: NMR δ_H 1.36 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.75-1.88 (2 H, m, C*H*$_2$CHO), 2.80 (1 H, m, CHC=C), 2.93 (1 H, m, CHC=C), 3.51 (1 H, dd, *J* = 11.7, 5.9 Hz, CH_2OBn), 3.53 (1 H, dd, *J* = 11.7, 5.9 Hz, CH_2OBn), 3.80 (3 H, s, OMe), 4.21 (1 H, ddd, *J* = 9.4, 5.9, 3.1 Hz, 4-H), 4.32 (1 H, q, *J* = 5.9 Hz, 5-H), 4.50 (1 H, d, *J* = 11.9 Hz, CHPh), 4.59 (1 H, d, *J* = 11.9 Hz, CHPh), 7.27-7.32 (5H, m, Ph-H_5), 7.43 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.47 (2 H, d, *J* = 9.0 Hz, Ar 3,5-H_2); MS *m/z* 488.1255 (M + H) ($C_{25}H_{29}{}^{81}BrNO_4$ requires 488.1259), 486.1263 (M + H) ($C_{25}H_{29}{}^{79}BrNO_4$ requires 486.1279), 430/428 (M - C_2H_3NO), 91 (Bn). Compound **24c** was treated with guanidine, as for the synthesis of **25a**, to give **25c** (57%) as a pale buff solid: mp 62-64°C; IR ν_{max} 3462, 1635 cm^{-1}; NMR δ_H 1.26 (6 H, s, Me_2), 1.58-1.63 (2 H, m, C*H*$_2$CHO), 2.22 (1 H, ddd, *J* = 13.3, 10.1, 5.9 Hz, Pyr-CH), 2.49 (1 H, ddd, *J* = 13.3, 10.1, 5.9 Hz, Pyr-CH), 3.41 (2 H, d, *J* = 5.9 Hz, CH_2OBn), 4.00 (1 H, ddd, *J* = 9.8, 5.9, 3.5 Hz, dioxolane 4-H), 4.20 (1 H, q, *J* = 5.9 Hz, dioxolane 5-H), 4.45 (1 H, d, *J* = 12.1 Hz, CHPh), 4.54 (1 H, d, *J* = 12.1 Hz, CHPh), 4.65 (2 H, br, NH_2), 5.06 (2 H, br, NH_2), 7.04 (1 H, d, *J* = 7.8 Hz, Ar 2-H), 7.06 (1 H, d, *J* = 8.2 Hz, Ar 6-H), 7.23-7.33 (5 H, m, Ph-H_5), 7.50 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2); MS *m/z* 515.1483 (M + H) ($C_{25}H_{30}{}^{81}BrN_4O_3$ requires 515.1480), 513.1497 (M + H) ($C_{25}H_{30}{}^{79}BrN_4O_3$ requires 513.1501), 499/497 (M – CH_3), 91 (Bn).

6.35. (4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(3,4-dichlorophenyl)-4-cyano-3-methoxybut-3-en-yl)-2,2-dimethyl-1,3-dioxolane (24d) and 6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(3,4-dichlorophenyl)pyrimidine-2,4-diamine (25d)

Compound **22d**/**23d** was treated with CH_2N_2, as for the synthesis of **24a**, to give **24d** (97%) as a pale yellow oil: NMR δ_H 1.36 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.77 (1 H, m, C*H*CHO), 1.85 (1 H, m, C*H*CHO), 2.81 (1 H, m, CHC=C), 2.94 (1 H, m, CHC=C), 3.51 (1 H, dd, *J* = 11.3, 6.0 Hz, CHOBn), 3.53 (1 H, dd, *J* = 11.3, 6.0 Hz, CHOBn), 3.84 (3 H, s, OMe), 4.21 (1 H, ddd, *J* = 9.4, 6.0, 3.1 Hz, 4-H), 4.32 (1 H, q, *J* = 6.0 Hz, 5-H), 4.49 (1 H, d, *J* = 12.1 Hz, CHPh), 4.58 (1 H, d, *J* = 12.1 Hz, CHPh), 7.26-7.32 (5H, m, Ph-H_5), 7.37 (1 H, d, *J* = 8.6 Hz, Ar 5-H), 7.42 (1 H, dd, *J* = 8.6, 2.1 Hz, Ar 6-H), 7.73 (1 H, d, *J* = 2.1 Hz, Ar 2-H); MS *m/z* 480.1319 (M + H) ($C_{25}H_{28}{}^{37}Cl_2NO_4$ requires 480.1336), 478.1344 (M + H) ($C_{25}H_{28}{}^{37}Cl\,{}^{35}ClNO_4$ requires 478.1365), 476.1367 (M + H) ($C_{25}H_{28}{}^{35}Cl_2NO_4$ requires 476.1395), 422/420/418 (M - C_2H_3NO), 91 (Bn). Compound **24d** was treated with guanidine, as for the synthesis of **25a**, to give **25d** (47%) as a pale buff solid: mp 67-69°C; IR ν_{max} 3411, 1637 cm^{-1}; NMR δ_H 1.27 (6 H, s, Me_2), 1.60 (1 H, m, C*H*CHO), 1.73 (1 H, m, C*H*CHO), 2.25 (1 H, m, Pyr-CH), 2.47 (1 H, m, Pyr-CH), 3.44 (2 H, d, *J* = 6.0 Hz, CH_2OBn), 3.99 (1 H, ddd, *J* = 9.8, 6.0, 3.5 Hz, dioxolane 4-H), 4.20 (1 H, q, *J* = 6.0 Hz, dioxolane 5-H), 4.47 (1 H, d, *J* = 12.3 Hz, CHPh), 4.56 (1 H, d, *J* = 12.3 Hz, CHPh), 4.85 (2 H, br, NH_2), 5.21 (2 H, br, NH_2), 7.03 (1 H, dd, *J* = 8.0, 2.0 Hz, Ar 6-H), 7.26-7.32 (5 H, m, Ph-H_5), 7.35 (1 H, d, *J* = 2.0 Hz, Ar 2-H), 7.46 (1 H, d, *J* = 8.0 Hz, Ar 5-H); MS *m/z* 507.1563 (M + H) ($C_{25}H_{29}{}^{37}Cl_2N_4O_3$ requires 507.1557), 505.1586 (M + H) ($C_{25}H_{29}{}^{37}Cl\,{}^{35}ClN_4O_3$ requires 505.1587), 503.1617 (M + H) ($C_{25}H_{29}{}^{35}Cl_2N_4O_3$ requires 503.1616), 91 (Bn).

6.36. 6-((3*R*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-phenylpyrimidine-2,4-diamine (26a)

Compound **25a** (700 mg, 1.6 mmol) was stirred for 16 h with aq. CF_3CO_2H (30%, 70 mL). Evaporation and chromatography ($CHCl_3$ / MeOH 7:3) to give **26a** (600 mg, 95%) as a highly hygroscopic pale yellow solid: NMR (CD_3OD) δ_H 1.69 (1 H, m, 2'-H), 1.88 (1 H, m, 2'-H), 2.43 (1 H, m, 1'-H), 2.60 (1 H, ddd, *J* = 15.3, 10.6, 5.5 Hz, 1'-H), 3.43-3.47 (2 H, m, 3',4'-H_2), 3.49-3.56 (2 H, m, 5'-H_2), 4.52 (1 H, d, *J* = 12.6 Hz, CHPh), 4.56 (1 H, d, *J* = 12.6 Hz, CHPh), 7.27-7.41 (6 H, m, 2 × Ph 3,4,5-H_3), 7.47-7.58 (4 H, m, 2 × Ph 2,6-H_2); MS *m/z* 417 (M + Na), 395.2097 (M + H) ($C_{22}H_{27}N_4O_3$ requires 395.2083), 243 (M - $C_9H_{11}O_2$), 213 (M – $C_{10}H_{13}O_3$), 91 (Bn).

6.37. 6-((3*R*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-(3-chlorophenyl)pyrimidine-2,4-diamine (26b)

Compound **25b** was treated with aq. CF_3CO_2H, as for the synthesis of **25a**, to give **26b** (87%) as a white solid: mp 131-133°C; NMR (CD_3OD) δ_H 1.64 (1 H, m, 2'-H), 1.82 (1 H, m, 2'-H), 2.37 (1 H, m, 1'-H), 2.51 (1 H, m, 1'-H), 3.49-3.55 (2 H, m, 3',4'-H_2), 3.57-3.64 (2 H, m, 5'-H_2), 4.52 (1 H, d, *J* = 12.5 Hz, CHPh), 4.56 (1 H, d, *J* = 12.5 Hz, CHPh), 7.24 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.33-7.37 (5 H, m, Ph-H_5), 7.47 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); NMR (CD_3OD) δ_C 29.02, 30.84, 71.03, 71.61, 72.09, 72.99, 107.42, 121.01, 127.36, 127.58, 128.01, 129.32, 132.16, 134.14, 138.10, 161.81, 162.16, 163.66; MS m/z 453/451 (M + Na), 431.1657 (M + H) ($C_{22}H_{26}{}^{37}ClN_4O_3$ requires 431.1663), 429.1680 (M + H) ($C_{22}H_{26}{}^{35}ClN_4O_4$ requires 429.1693), 279/277 (M - $C_9H_{11}O_2$), 249/247 (M – $C_{10}H_{13}O_3$), 91 (Bn).

6.38. 6-((3*R*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine-2,4-diamine (26c)

Compound **25c** was treated with aq. CF_3CO_2H, as for the synthesis of **25a**, to give **26c** (91%) as a highly hygroscopic pale buff solid: NMR δ_H 1.65-1.84 (2 H, m, 2'-H_2), 2.35-2.55 (2 H, m, 1'-H_2), 3.52-3.71 (4 H, m, 3',4',5'-H_4), 4.49 (1 H, d, *J* = 12.1 Hz, CHPh), 4.54 (1 H, d, *J* = 12.1 Hz, CHPh), 4.71 (2 H, br, NH_2), 5.09 (2 H, br, NH_2), 7.05 (2 H, d, *J* = 8.4 Hz, Ar 2,6-H_2), 7.24-7.34 (5 H, m, Ph-H_5), 7.54 (2 H, d, *J* = 8.4 Hz, Ar 3,5-H_2); MS *m/z* 497/495 (M + Na), 475.1184 (M + H) ($C_{22}H_{26}{}^{81}BrN_4O_3$ requires 475.1167), 473.1179 (M + H) ($C_{22}H_{26}{}^{79}BrN_4O_4$ requires 473.1188), 323/321 (M - $C_9H_{11}O_2$), 293/291 (M – $C_{10}H_{13}O_3$), 91 (Bn).

6.39. 6-((3*R*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-(3,4-dichlorophenyl)pyrimidine-2,4-diamine (26d)

Compound **25d** was treated with aq. CF_3CO_2H, as for the synthesis of **25a**, to give **26d** (74%) as a pale yellow solid: mp 123-125°C; NMR (CD_3OD) δ_H 1.60 (1 H, m, 2'-H), 1.82 (1 H, m, 2'-H), 2.31 (1 H, ddd, *J* = 14.2, 9.0, 5.9 Hz, 1'-H), 2.47 (1 H, ddd, *J* = 14.2, 9.0, 5.9 Hz, 1'-H), 3.44-3.51 (2 H, m, 3',4'-H_2), 3.54-3.59 (2 H, m, 5'-H_2), 4.47 (1 H, d, *J* = 14.1 Hz, CHPh), 4.52 (1 H, d, *J* = 14.1 Hz, CHPh), 7.15 (1 H, dd, *J* = 8.2, 1.9 Hz, Ar 6-H), 7.22-7.33 (5 H, m, Ph-H_5), 7.41 (1 H, d, *J* = 1.9 Hz, Ar 2-H), 7.57 (1 H, d, *J* = 8.2 Hz, Ar 5-H); NMR (CD_3OD) δ_C 23.30, 30.97, 71.23, 71.58, 72.99, 106.22, 181.16, 127.31, 127.55, 127.99, 130.64, 131.15,

131.95, 132.61, 134.54, 138.17, 162.09, 162.83, 163.16; MS *m/z* 489/487/485 (M + Na), 467.1258 (M + H) ($C_{22}H_{25}{}^{37}Cl_2N_4O_3$ requires 467.1244), 465.1272 (M + H) ($C_{22}H_{25}{}^{37}Cl\,{}^{35}ClN_4O_3$ requires 465.1274), 463.1293 (M + H) ($C_{22}H_{25}{}^{35}Cl_2N_4O_3$ requires 463.1303), 315/313/311 (M - $C_9H_{11}O_2$), 91 (Bn).

6.40. (4*R*,5*S*)-4-(4-Cyano-3-oxo-4-phenylbutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27a) / (4*R*,5*S*)-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28a)

$LiN(SiMe_3)_2$ (1.0 M in THF, 30 mL, 30 mmol) was added to phenylacetonitrile (1.75 g, 15 mmol) in dry Et_2O (15 mL) under N_2 at -78°C. After 10 min, **20** (3.5 g, 15 mmol) was added. The mixture was warmed to 20°C and was stirred for 72 h. Water was added. The solution was washed twice (Et_2O) before being acidified to pH 6 with aq. citric acid (1 M) in the presence of EtOAc. The EtOAc phase was separated and washed with water. Drying, evaporation and chromatography (EtOAc / hexane, 3:1) gave **27a**/**28a** (450 mg, 10%) as a pale yellow oil: NMR δ_H 1.30 (3 H, s, Me), 1.40 (3 H, s, Me), 1.74-1.81 (2 H, m, C*H*$_2$CHO), 2.71 (1 H, m, CHC=O), 2.83 (1 H, m, CHC=O), 3.62 (2 H, d, *J* = 5.5 Hz, CH_2OH), 4.07 (1 H, m, 4-H), 4.15 (1 H, m, 5-H), 4.79 (1 H, s, CHCN), 7.36-7.50 (5 H, m, Ph-H_5); MS *m/z* 303.1464 (M + H) ($C_{17}H_{21}NO_4$ requires 303.1470), 287 (M - CH_3), 271 (M - CH_3O), 245 (M – C_2H_3NO).

6.41. (4*R*,5*S*)-4-(4-(4-Chlorophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27b) / (4*R*,5*S*)-4-(4-(4-chlorophenyl)-4-cyano-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28b)

4-Chlorophenylacetonitrile was condensed with **20**, as for the synthesis of **27a**/**28a**, to give **27b**/**28b** (7%) as a pale yellow solid: mp 133-135°C; NMR δ_H 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.35-2.52 (2 H, m, C*H*$_2$CHO), 2.66-2.84 (2 H, m, CH_2C=O), 3.63 (1 H, dd, *J* = 12.7, 4.9 Hz, C*H*OH), 3.73 (1 H, dd, *J* = 12.7, 4.9 Hz, C*H*OH), 4.21-4.28 (2 H, m, 4,5-H_2), 7.35 (2 H, d, *J* = 9.0 Hz, Ar 2,6-H_2), 7.44 (2 H, d, *J* = 9.0 Hz, Ar 3,5-H_2); MS *m/z* 340 (M + H) $C_{17}H_{20}{}^{37}ClNO_4$, 338 (M + H) $C_{17}H_{20}{}^{35}ClNO_4$, 322/320 (M – OH), 308/306 (M - CH_3O).

6.42. ((4*R*,5*S*)-4-(4-(4-Bromophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27c) / (4*R*,5*S*)-4-(4-(4-bromophenyl)-4-cyano-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28c)

4-Bromophenylacetonitrile was condensed with **20**, as for the synthesis of **27a**/**28a**, to give

27c/**28c** (6%) as a pale yellow solid: mp 128-130°C; NMR δ_H 1.41 (3 H, s, Me), 1.51 (3 H, s, Me), 2.39-2.48 (2 H, m, C*H*$_2$CHO), 2.69-2.79 (2 H, m, CH$_2$C=O), 3.62 (1 H, dd, *J* = 11.6, 5.3 Hz, C*H*OH), 3.73 (1 H, dd, *J* = 11.6, 5.3 Hz, C*H*OH), 4.19-4.27 (2 H, m, 4,5-H$_2$), 7.30 (2 H, d, *J* = 8.5 Hz, Ar 2,6-H$_2$), 7.47 (2 H, d, *J* = 8.5 Hz, Ar 3,5-H$_2$); MS *m/z* 382.0480 (M + H) ($C_{17}H_{20}{}^{81}BrNO_4$ requires 382.0476), 380.0475 (M + H) ($C_{17}H_{20}{}^{79}BrNO_4$ requires 380.0497), 368/366 (M - CH$_3$), 342/340 (M - C_2H_3N), 326/324 (M – C_2H_3NO).

6.43. (4*R*,5*S*)-4-(4-Cyano-4-(3,4-dichlorophenyl)-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27d) / (4*R*,5*S*)-4-(4-cyano-4-(3,4-dichlorophenyl)-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28d)

3,4-Dichlorophenylacetonitrile was condensed with **20**, as for the synthesis of **27a**/**28a**, to give **27d**/**28d** (7%) as a pale yellow solid: mp 117-118 °C; IR ν_{max} 3424, 2209, 1718 cm^{-1}; NMR δ_H 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.38-2.47 (2 H, m, C*H*$_2$CHO), 2.72-2.84 (2 H, m, CH$_2$C=O), 3.64 (1 H, dd, *J* = 11.5, 5.5 Hz, C*H*OH), 3.75 (1 H, dd, *J* = 11.5, 5.5 Hz, C*H*OH), 4.18-4.29 (2 H, m, 4,5-H$_2$), 7.27 (1 H, dd, *J* = 8.5, 2.2 Hz, Ar 6-H), 7.43 (1 H, d, *J* = 8.5 Hz, Ar 5-H), 7.54 (1 H, d, *J* = 2.2 Hz, Ar 2-H). MS *m/z* 375/373/371 (M + H), 334/332/330 (M - C_2H_2N), 316/314/312 (M – C_2H_3NO).

6.44. (4*R*,5*S*)-4-(4-Cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29a) and 6-(2-((4*R*,5*S*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (30a)

Compound **27a**/**28a** was treated with CH_2N_2, as for the synthesis of **24a**, to give **29a** (330 mg, 95%) as a pale yellow oil: NMR δ_H 1.40 (3 H, s, 2-Me), 1.51 (3 H, s, 2-Me), 1.87-2.01 (2 H, m, C*H*$_2$CHO), 2.84-3.02 (2 H, m, CH$_2$C=C), 3.69 (2 H, d, *J* = 5.9 Hz, C*H*$_2$OH), 3.86 (3 H, s, OMe), 4.22-4.27 (2 H, m, 4,5-H$_2$), 7.24-7.42 (5 H, m, Ph-H$_5$); MS *m/z* 318.1707 (M + H) ($C_{18}H_{24}NO_4$ requires 318.1705), 302 (M – Me), 277 (M - C_2H_2N), 258 (M – C_2H_5NO). Compound **29a** was treated with guanidine, as for the synthesis of **25a** (reaction time 4 h, chromatographic eluant $CHCl_3$ / MeOH (9:1)), to give **30a (**42%) as a pale buff solid: mp 72-75°C; NMR (D_2O) δ_H 1.27 (3 H, s, Me), 1.31 (3 H, s, Me), 1.75-1.88 (2 H, m, C*H*$_2$CHO), 2.32 (1 H, ddd, *J* = 15.9, 9.7, 6.0 Hz, Pyr-CH), 2.49 (1 H, ddd, *J* = 15.9, 8.7, 7.4 Hz, Pyr-CH), 3.53 (1 H, dd, *J* = 11.6, 5.9 Hz, C*H*OH), 3.65 (1 H, dd, *J* = 11.6, 5.9 Hz, C*H*OH), 4.05 (1 H, dd, *J* = 12.2, 5.9 Hz, dioxolane 4-H), 4.12 (1 H, q, *J* = 5.9 Hz, dioxolane 5-H), 4.63 (2 H, br, NH$_2$), 4.95 (2 H, br, NH$_2$), 7.21 (1 H, d, *J* = 7.9 Hz, Ar 2-H), 7.22 (1 H, d, *J* = 7.9 Hz, Ar 6-H), 7.36

(1 H, t, *J* = 7.9 Hz, Ar 4-H), 7.21 (2H, t, *J* = 7.9 Hz, Ar 3,5-H_2); NMR (D_2O) δ_C 23.01, 25.58, 25.70, 28.17, 58.60, 74.07, 75.63, 105.27, 106.26, 125.50, 126.82, 126.85, 127.94, 158.71, 159.82, 162.92; MS *m/z* 345.1935 (M + H) ($C_{19}H_{24}N_4O_3$ requires 345.1926), 329 (M - Me), 200 (M – $C_7H_{12}O_3$).

6.45. (4*R*,5*S*)-4-(4-(4-Chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29b) and 5-(4-chlorophenyl)-6-(2-((4*R*,5*S*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30b)

Compound **27b**/**28b** was treated with CH_2N_2, as for the synthesis of **24a**, to give **29b** (90%) as a pale yellow oil. Compound **29b** was treated with guanidine, as for the synthesis of **25a** (reaction time 10 h, chromatographic eluant $CHCl_3$ / MeOH (9:1)), to give **30b** (12%) as a white solid: mp 94-95°C; NMR δ_H 1.27 (3 H, s, Me), 1.30 (3 H, s, Me) 1.73 (1 H, m, C*H*CHO), 1.84 (1 H, m, C*H*CHO), 2.29 (1 H, ddd, *J* = 13.4, 10.8, 5.1 Hz, Pyr-CH), 2.46 (1 H, ddd, *J* = 13.4, 10.4, 5.9 Hz, Pyr-CH), 3.56 (1 H, dd, *J* = 11.7, 6.1 Hz, C*H*OH), 3.66 (1 H, dd, *J* = 11.7, 6.1 Hz, C*H*OH), 4.02 (1 H, dt, *J* = 8.1, 5.8 Hz, dioxolane 4-H), 4.13 (1 H, q, *J* = 5.8 Hz, dioxolane 5-H), 5.04 (2 H, br, NH_2), 5.76 (2 H, br, NH_2), 7.16 (2 H, d, *J* = 7.0 Hz, Ar 2,6-H_2), 7.42 (2 H, d, *J* = 7.0 Hz, Ar 3,5-H_2); NMR δ_C 25.57, 28.04, 28.42, 30.46, 60.90, 76.77, 77.09, 107.39, 107.94, 129.71, 132.64, 134.29, 160.82, 162.48, 164.40; MS *m/z* 381.1506 (M + H) ($C_{18}H_{24}{}^{37}ClN_4O_3$ requires 381.1507), 379.1525 (M + H) ($C_{18}H_{24}{}^{35}ClN_4O_4$ requires 379.1536), 236/234 (M - $C_7H_{12}O_3$), 188/186 (M – $C_8H_{13}ClO_3$).

6.46. (4*R*,5*S*)-4-(4-(4-Bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29c) and 5-(4-bromophenyl)-6-(2-((4*R*,5*S*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30c)

Compound **27c**/**28c** was treated with CH_2N_2, as for the synthesis of **24a**, to give **29c** (90%) as a pale yellow oil: IR ν_{max} 3435, 2243, 1592 cm^{-1}; NMR δ_H 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.38-2.49 (2 H, m, CH_2CHO), 2.74-2.80 (2 H, m, CH_2C=C), 3.33 (3 H, s, OMe), 3.64 (1 H, dd, *J* = 11.1, 5.0 Hz, C*H*OH), 3.74 (1 H, dd, *J* = 11.1, 5.0 Hz, C*H*OH), 4.21-4.27 (2 H, m, dioxolane 4,5-H_2), 7.29 (2 H, d, *J* = 8.5 Hz, Ar 2,6-H_2), 7.50 (2 H, d, *J* = 8.5 Hz, Ar 3,5-H_2). Compound **29c** was treated with guanidine, as for the synthesis of **25a** (reaction time 10 h, chromatographic eluant $CHCl_3$ / MeOH (9:1)), to give **30c** (20%) as a white solid: mp 124-125°C; NMR δ_H 1.27 (6 H, s, Me_2), 1.61-1.79 (2 H, m, CH_2CHO), 2.26 (1 H, ddd, *J* = 13.0, 10.6, 5.8 Hz, Pyr-CH), 2.46 (1 H, ddd, *J* = 13.0, 10.6, 5.8 Hz, Pyr-CH), 3.50 (1 H, dd, *J* =

11.1, 5.9 Hz, C*H*OH), 3.54 (1 H, dd, *J* = 11.1, 5.9 Hz, C*H*OH), 4.01 (1 H, ddd, *J* = 9.9, 5.9, 3.9 Hz, dioxolane 4-H), 4.08 (1 H, q, *J* = 5.9 Hz, dioxolane 5-H), 7.21 (2 H, d, *J* = 7.7 Hz, Ar 2,6-H_2), 7.66 (2 H, d, *J* = 7.7 Hz, Ar 3,5-H_2); NMR (CD_3OD) δ_C 24.43, 27.11, 28.49, 30.66, 60.32, 76.63, 77.89, 107.20, 107.82, 121.84, 132.21, 132.57, 133.50, 160.82, 162.98, 163.71; MS *m/z* 425.1007 (M + H) ($C_{18}H_{24}{}^{81}BrN_4O_3$ requires 425.1011), 423.1019 (M + H) ($C_{18}H_{24}{}^{79}BrN_4O_4$ requires 423.1013), 280/278 (M - $C_7H_{12}O_3$), 186 (M – $C_8H_{13}BrO_3$).

6.47. (4*R*,5*S*)-4-(4-(3,4-Dichlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29d) and 5-(3,4-dichlorophenyl)-6-(2-((4*R*,5*S*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30d)

Compound **27c**/**28c** was treated with CH_2N_2, as for the synthesis of **24a**, to give **29d** (91%) as a pale yellow oil: IR ν_{max} 3467, 2210, 1597 cm^{-1}; NMR δ_H 1.40 (3 H, s, 2-Me), 1.50 (3 H, s, 2-Me), 2.30-2.40 (2 H, m, CH_2CHO), 2.72-2.80 (2 H, m, CH_2C=C), 3.37 (3 H, s, OMe), 3.58-3.74 (2 H, m, CH_2OH), 4.18-4.26 (2 H, m, 4,5-H_2), 7.51 (1 H, d, *J* = 8.3 Hz, Ar 5-H), 7.84 (1 H, dd, *J* = 8.3, 2.0 Hz, Ar 6-H), 8.10 (1 H, d, *J* = 2.0 Hz, Ar 2-H). Compound **29d** was treated with guanidine, as for the synthesis of **25a** (reaction time 6 h, chromatographic eluant $CHCl_3$ / MeOH (9:1)), to give **30d** (9%) as a white solid: mp 114-115°C; NMR δ_H 1.29 (3 H, s, Me), 1.33 (3 H, s, Me), 1.78 (1 H, m, C*H*CHO), 1.88 (1 H, m, C*H*CHO), 2.34 (1 H, m, Pyr-CH), 2.48 (1 H, m, Pyr-CH), 3.58 (1 H, dd, *J* = 11.4, 5.9 Hz, CHOH), 3.67 (1 H, dd, *J* = 11.4, 5.9 Hz, CHOH), 4.05 (1 H, m, dioxolane 4-H), 4.15 (1 H, q, *J* = 5.6 Hz, dioxolane 5-H), 4.95 (2 H, br, NH_2), 5.53 (2 H, br, NH_2), 7.08 (1 H, dd, *J* = 8.4, 1.7 Hz, Ar 6-H), 7.38 (1 H, d, *J* = 1.7 Hz, Ar 2-H), 7.52 (1 H, d, *J* = 8.4 Hz, Ar 5-H); NMR δ_C 25.53, 28.04, 28.12, 30.35, 61.05, 65.83, 70.51, 106.62, 108.04, 130.15, 131.41, 131.45, 133.51, 134.4, 160.93, 162.22, 168.33; MS *m/z* 417.1091 (M + H) ($C_{18}H_{23}{}^{37}Cl_2N_4O_3$ requires 417.1088), 415.1103 (M + H) ($C_{18}H_{23}{}^{37}Cl\ {}^{35}ClN_4O_3$ requires 415.1117), 413.1129 (M + H) ($C_{18}H_{23}{}^{35}Cl_2N_4O_3$ requires 413.1147), 272/270/268 (M - $C_7H_{12}O_3$) 186 (M – $C_8H_{12}Cl_2O_3$).

6.48. Diethyl (*R*,*R*)-2,2-diethyl-1,3-dioxolane-4,5-dicarboxylate (32)

Diethyl (*R*,*R*)-2,3-dihydroxybutanedioate **31** (15.0 g, 70 mmol), 2,2-dimethoxypropane (8.0 g, 80 mmol) and 4-methylbenzenesulfonic acid (66 mg, 0.34 mmol) in dichloromethane (200 mL) were heated under reflux through activated 4 Å molecular sieves (33 g) in a Soxhlet apparatus for 3 h. Na_2CO_3 (83 mg, 1.0 mmol) was added. Filtration, drying and evaporation gave

32 (16.0 g, 89%) as a pale buff oil (lit.[47] oil): NMR δ_H 1.32 (6 H, t, *J* = 7.2 Hz, 2 × CH_2CH_3), 1.50 (6 H, s, CMe_2), 4.28 (4 H, q, *J* = 7.2 Hz, 2 × CH_2), 4.77 (2 H, s, 4,5-H_2).

6.49. (*S*,*S*)-4,5-Di(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane (33)

$LiAlH_4$ (6.0 g, 150 mmol) was heated in dry THF (60 mL) for 30 min. Compound **32** (18.0 g, 70 mmol) in dry THF (80 mL) was added during 1.5 h. The mixture was heated under reflux for 5 h, then cooled to 0ºC. Water (10 mL), aq. NaOH (4 M, 10 mL) and water (30 mL) were added. Filtration and evaporation gave **33**. The solid was extracted with hot 1,4-dioxane; evaporation gave further **33** (total 7.0 g, 60%) as a pale yellow oil (lit.[48] oil): NMR δ_H 1.41 (6 H, s, Me_2), 2.65 (2 H, br, 2 × OH), 3.68-3.78 (4 H, m, 2 × CH_2), 3.97 (2 H, m, 4,5-H_2).

6.50. (*S*,*S*)-4,5-Di(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane (34) and (*S*,*S*)-4-benzyloxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (35)

NaH (60% oil, 1.4 g, 34 mmol) was stirred in dry DMF (20 mL) under N_2 for 30 min. Compound **33** (5.0 g, 31 mmol) in DMF (20 mL) was added dropwise and the mixture was stirred for 30 min before BnCl (4.0 g, 32 mmol) was added. The mixture was stirred for 1.5 h, then poured into ice-water (250 mL) and extracted thrice with Et_2O. The combined extracts were washed with water and brine. Drying, evaporation and chromatography (hexane / Et_2O 1:1) gave **34** (2.2 g, 28%) as a pale yellow oil (lit.[49] oil): NMR δ_H 1.42 (6 H, s, Me_2), 3.54-3.66 (4 H, m, 2 × CH_2OBn), 4.02 (2 H, m, 4,5-H_2), 4.54 (2 H, d, *J* = 12.3 Hz, 2 × CHPh), 4.58 (2 H,d, *J* = 12.3 Hz, 2 × CHPh), 7.35 (10 H, m, 2 × Ph-H_5). Further elution gave **35** (3.2 g, 64%) as a pale yellow oil. $[\alpha]^{20}_D$ = +8.0° (c 3.2, $CHCl_3$) (lit.[50] $[\alpha]^{23}_D$ = +8.2° (c 1.0, $CHCl_3$)); NMR δ_H 1.41 (3 H, s, Me), 1.42 (3 H, s, Me), 2.33 (1 H, dd, *J* = 8.6, 4.3 Hz, OH), 3.55 (1 H, dd, *J* = 9.8, 4.3 Hz, CHOBn), 3.64-3.70 (2 H, m, C*H*OH + CHOBn), 3.75 (1 H, dt, *J* = 11.7, 4.3 Hz, C*H*OH), 3.94 (1H, dt, *J* = 8.3, 4.3 Hz, 5-H), 4.05 (1 H, dt, *J* = 8.3, 4.3 Hz, 4-H), 4.58 (2 H, s, CH_2Ph), 7.29-7.35 (5H, m, Ph-H_5).

6.51. (4*S*,5*R*)-4-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-carboxaldehyde (4-O-benzyl-2,3-O-isopropylidene-L-threose) (36)

Compound **35** (3.6 g, 14 mmol) was stirred with pyridinium chlorochromate (3.6 g, 35 mmol), NaOAc (300 mg, 3.5 mmol) and powdered 4 Å molecular sieves (3.0 g) in CH_2Cl_2 (215 mL) under N_2 for 3 h. The mixture was passed through a bed of silica. The silica was extracted with Et_2O. Evaporation of the solvent from the combined filtrate and extract gave **36**

(3.3 g, 93%) as a pale yellow oil: $[\alpha]^{20}_D$ = +14° (c 3, $CHCl_3$) (lit.[51] $[\alpha]^{20}_D$ = +16.2° (c 1, $CHCl_3$); NMR δ_H 1.43 (3 H, s, Me), 1.50 (3 H, s, Me), 3.67 (2 H, d, *J* = 4.0 Hz, CH_2OBn), 4.19-4.29 (2 H, m, 4,5-H_2), 4.58 (1 H, d, *J* = 10.5 Hz, CHPh), 4.61 (1 H, d, *J* = 10.5 Hz, CHPh), 7.25-7.36 (5 H, m, Ph-H_5), 9.76 (1 H, d, *J* = 1.5 Hz, CHO).

6.52. Ethyl (*Z*,4*S*,5*S*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (37*Z*) and ethyl (*E*,4*S*,5*S*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (37*E*)

Compound **36** (2.0 g, 8.0 mmol), ethyl triphenylphosphoranylideneacetate (4.2 g, 16 mmol) and benzoic acid (50 mg, 0.4 mmol) were heated at reflux in PhMe (200 mL) under N_2 for 4 h. The evaporation residue was extracted thrice with Et_2O. Evaporation and chromatography (hexane / Et_2O 5:1) gave **37*Z*** (800 g, 31%) as a colourless oil (lit.[52] oil): NMR δ_H 1.25 (3 H, t, *J* = 7.1 Hz, CH_2CH_3), 1.45 (6 H, s, CMe_2), 3.68 (2 H, d, *J* = 3.1 Hz, CH_2OBn), 3.97 (1 H, m, 4-H), 4.12 (2 H, q, *J* = 7.1 Hz, , CH_2Me), 4.56 (1 H, d, *J* = 12.1 Hz, CHPh), 4.62 (1 H, d, *J* = 12.1 Hz, CHPh), 5.38 (1 H, td, *J* = 8.3, 1.2 Hz, 5-H), 5.92 (1 H, dd, *J* = 11.7, 1.2 Hz, $CHCO_2$), 6.18 (1 H, dd, *J* = 11.7, 8.3 Hz, $CH{=}CCO_2$), 7.32-7.37 (5H, m, Ph-H_5). Further elution gave **37*E*** (800 mg, 31%) as a colourless oil (lit.[53] oil): NMR δ_H 1.29 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.43 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 3.62 (2 H, d, *J* = 4.7 Hz, CH_2OBn), 3.95 (1 H, dt, *J* = 8.6, 4.7 Hz, 4-H), 4.19 (2 H, q, *J* = 7.0 Hz, CH_2Me), 4.42 (1 H, ddd, *J* = 8.6, 5.5, 1.4 Hz, 5-H), 4.56 (1 H, *J* = 12.1 Hz, CHPh), 4.61 (1 H, d, *J* = 12.1 Hz, CHPh), 6.09 (1 H, dd, *J* = 15.6, 1.4 Hz, $CHCO_2$), 6.88 (1 H, dd, *J* = 15.6, 5.5 Hz, $CH{=}CCO_2$), 7.27-7.36 (5 H, m, Ph-H_5).

6.53. Ethyl (4*S*,5*S*)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propanoate (38)

A mixture of **37*Z*** and **37*E*** (620 mg, 1.9 mmol) was stirred in EtOH (25 mL) with Pd/C (5%, 30 mg) under H_2 for 1 h. Filtration (Celite®), evaporation and chromatography (hexane / Et_2O 4:1) gave **38** (400 mg, 63%) as a pale yellow oil: $[\alpha]^{20}_D$ = -15° (c 4.0, $CHCl_3$); NMR δ_H 1.23 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 1.38 (3 H, s, 2-Me), 1.39 (3 H, s, 2-Me), 1.84 (1 H, m, $CHCH_2CO_2$), 1.96 (1 H, m, $CHCH_2CO_2$), 2.37-2.54 (2 H, m, CH_2CO_2), 3.53-3.60 (2 H, m, CH_2OBn), 3.80-3.87 (2 H, m, 4,5-H_2), 4.12 (2 H, q, *J* = 7.0 Hz, , CH_2Me), 4.56 (1 H, d, *J* = 12.3 Hz, CHPh), 4.59 (1 H, d, *J* = 12.3 Hz, CHPh), 7.32-7.34 (5 H, m, Ph-H_5); MS *m/z* 323.1856 (M + H) ($C_{19}H_{26}O_5$ requires 323.1858), 265 (M – C_3H_5O), 91 (Bn).

6.54. (4*S*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane (39a) / (4*S*,5*S*)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (40a)

Phenylacetonitrile was condensed with **38**, as for the synthesis of **22a**/**23a**, to give **39a**/**40a** (14%) as a pale yellow solid: mp 75-77ºC; IR ν_{max} 2206, 1731 cm^{-1}; NMR δ_H 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.86 (1 H, m, C*H*CHO), 2.00 (1 H, m, C*H*CHO), 2.48-2.65 (2 H, m, CH_2C=O), 3.54-3.63 (2 H, m, 4,5-H_2), 3.87-3.89 (2 H, m, CH_2OBn), 4.57 (1 H, d, *J* = 12.5 Hz, CHPh), 4.61 (1 H, d, *J* = 12.5 Hz, CHPh), 5.59 (1 H, s, CHCN), 7.33-7.64 (8 H, m, Ph' 3,4,5-H_3 + Ph-H_5), 8.11 (2 H, d, *J* = 7.0 Hz, Ph 2,6-H_2); MS *m/z* 392.1859 (M - H) ($C_{24}H_{26}NO_4$ requires 392.1861), 335 (M - C_2H_4NO), 317 (M – C_7H_6), 91 (Bn).

6.55. (4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (39b) / (4*S*,5*S*)-5-benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-hydroxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (40b)

4-Chlorophenylacetonitrile was condensed with **38**, as for the synthesis of **22a**/**23a** (chromatographic eluant EtOAc / hexane (1:1)), to give **39b**/**40b** (41%) as a yellow oil: IR ν_{max} 2209, 1731 cm^{-1}; NMR δ_H 1.39 (3 H, s, Me), 1.40 (3 H, s, Me), 1.85 (1 H, m, C*H*CHO), 2.00 (1 H, m, C*H*CHO), 2.46-2.63 (2 H, m, CH_2C=O), 3.53-3.62 (2 H, m, 4,5-H_2), 3.83-3.87 (2 H, m, CH_2OBn), 4.57 (1 H, d, *J* = 12.1 Hz, CHPh), 4.60 (1 H, d, *J* = 12.1 Hz, CHPh), 7.26-7.40 (5H, m, Ph-H_5), 7.44 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2), 8.02 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2); MS *m/z* 430.1608 (M + H) ($C_{24}H_{27}{}^{37}ClNO_4$ requires 430.1599), 428.1623 (M + H) ($C_{24}H_{27}{}^{35}ClNO_4$ requires 428.1628), 372/370 (M – C_2H_3NO), 91 (Bn).

6.56. (4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (39c) / (4*S*,5*S*)-5-benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-hydroxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (39c)

4-Bromophenylacetonitrile was condensed with **38**, as for the synthesis of **22a**/**23a**, to give **39c**/**40c** (24%): as a yellow oil: IR ν_{max} 2208, 1718 cm^{-1}; NMR δ_H 1.33 (3 H, s, Me), 1.34 (3 H, s, Me), 1.75 (1 H, m, C*H*CHO), 1.91 (1 H, m, C*H*CHO), 2.69 (1 H, m, CHC=O), 2.79 (1 H, m, CHC=O), 3.55-3.65 (2 H, m, CH_2OBn), 3.93 (1 H, m, 5-H), 4.03 (1 H, dt, *J* = 8.0, 3.7 Hz, 4-H), 4.53 (1 H, d, *J* = 12.0 Hz, CHPh), 4.61 (1 H, d, *J* = 12.0 Hz, CHPh), 7.22 (2 H, d, *J* = 8.1 Hz, Ar 2,6-H_2), 7.26-7.36 (5H, m, Ph-H_5), 7.53 (2 H, d, *J* = 8.8 Hz, Ar 3,5-H_2); MS *m/z*

474.1103 (M + H) ($C_{24}H_{27}{}^{81}BrNO_4$ requires 474.1102), 472.1103 (M + H) ($C_{24}H_{27}{}^{79}BrNO_4$ requires 472.1123), 415/413 (M – C_2H_4NO), 91 (Bn).

6.57. (4*S*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41a) and 6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (42a)

Compound **39a**/**40a** was treated with CH_2N_2, as for the synthesis of **24a**, to give **41a** (79%) as a pale yellow oil: IR ν_{max} 2208, 1605 cm^{-1}; NMR δ_H 1.45 (3 H, s, Me), 1.49 (3 H, s, Me), 1.83 (1 H, m, C*H*CHO), 1.98 (1 H, m, C*H*CHO), 2.40-2.59 (2 H, m, CH_2C=C), 3.55-3.60 (2 H, m, 4,5-H_2), 3.75 (3 H, s, OMe), 3.81-3.85 (2 H, m, CH_2OBn), 4.55 (1 H, d, *J* = 12.7 Hz, CHPh), 4.58 (1 H, d, *J* = 12.7 Hz, CHPh), 7.14-7.45 (10 H, m, 2 × Ph-H_5); MS *m/z* 408.2184 (M + H) ($C_{25}H_{30}NO_4$ requires 408.2174), 391 (M - CH_4), 380 (M – HCN), 91 (Bn). Compound **41a** was condensed with guanidine, as for the synthesis of **25a** (reaction time 4 h, chromatographic eluant $CHCl_3$ / MeOH (9:1)), to give **42a** (67%) as a highly hygroscopic pale yellow solid: IR ν_{max} 3454, 1664 cm^{-1}; NMR δ_H 1.26 (3 H, s, Me), 1.29 (3 H, s, Me), 1.71-1.91 (2 H, m, C*H*$_2$CHO), 2.33 (1 H, ddd, *J* = 13.5, 10.3, 5.8 Hz, Pyr-CH), 2.45 (1 H, ddd, *J* = 13.5, 10.5, 5.7 Hz, PyR-CH), 3.44 (2 H, d, *J* = 4.6 Hz, CH_2OBn), 3.67 (1 H, dt, *J* = 7.9, 4.6 Hz, dioxolane 4-H), 3.75 (1 H, q, *J* = 4.6 Hz, dioxolane 5-H), 4.28 (1 H, d, *J* = 12.1 Hz, CHPh), 4.54 (1 H, d, *J* = 12.1 Hz, CHPh), 4.64 (2 H, br, NH_2), 5.14 (2 H, br, NH_2), 7.19-7.43 (10 H, m, 2 × Ph-H_5); MS *m/z* 435.2398 (M + H) ($C_{25}H_{31}N_4O_3$ requires 435.2396), 327 (M – C_7H_7O), 91 (Bn).

6.58. (4*S*,5*S*)-5-Benzyloxymethyl-4-(-4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41b) and 6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)ethyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (42b)

Compound **39b**/**40b** was treated with CH_2N_2, as for the synthesis of **24a**, to give **41b** (91%) as a pale yellow oil: NMR δ_H 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.62-1.75 (2 H, m, C*H*$_2$CHO), 2.86-2.96 (2 H, m, CH_2C=C), 3.52-3.65 (2 H, m, 4,5-H_2), 3.81 (3 H, s, OMe), 3.84-3.93 (2 H, m, CH_2OBn), 4.54 (1 H, d, *J* = 11.1 Hz, CHPh), 4.58 (1 H, d, *J* = 11.1 Hz, CHPh), 7.24-7.38 (9H, m, Ph-H_5 + Ar-H_4). Compound **41b** was condensed with guanidine, as for the synthesis of **25a** (reaction time 4 h, chromatographic eluant CH_2Cl_2/MeOH (4:1)), to give **42b** (48%) as a highly hygroscopic pale yellow solid: IR ν_{max} 3475, 3414, 1618 cm^{-1}; NMR δ_H 1.28 (3 H, s, Me), 1.31 (3 H, s, Me), 1.73 (1 H, m, C*H*CHO), 1.83 (1 H, m, C*H*CHO), 2.30 (1 H, ddd, *J* =

13.5, 10.5, 5.7 Hz, Pyr-CH), 2.45 (1 H, ddd, *J* = 13.5, 10.5, 5.7 Hz, Pyr-CH), 3.41-3.49 (2 H, m, CH_2OBn), 3.66 (1 H, dt, *J* = 8.2, 3.5 Hz, dioxolane 4-H), 3.51 (1 H, m, dioxolane 5-H), 4.50 (1 H, d, *J* = 12.1 Hz, CHPh), 4.54 (1 H, d, *J* = 12.1 Hz, CHPh), 4.69 (2 H, br, NH_2), 5.11 (2 H, br, NH_2), 7.10-7.35 (9 H, m, Ph-H_5 + Ar-H_4); MS *m/z* 471.1979 (M + H) ($C_{25}H_{30}{}^{37}ClN_4O_3$ requires 471.1976), 469.1999 (M + H) ($C_{25}H_{30}{}^{35}ClN_4O_3$ requires 469.2006), 363/361 (M – C_7H_7O), 91 (Bn).

6.59. (4*S*,5*S*)-5-Benzyloxymethyl-4-(-4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41c) and 6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)-ethyl)-5-(4-bromophenyl)pyrimidine-2,4-diamine (42c)

Compound **39c**/**40c** was treated with CH_2N_2, as for the synthesis of **24a**, to give **41c** (93%) as a pale yellow oil: NMR δ_H 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.78-1.86 (2 H, m, C*H*$_2$CHO), 2.85-2.95 (2 H, m, CH_2C=C), 3.52-3.68 (2 H, m, 4,5-H_2), 3.81 (3 H, s, OMe), 3.84-3.94 (2 H, m, CH_2OBn), 4.54 (1 H, d, *J* = 12.1 Hz, CHPh), 4.59 (1 H, d, *J* = 12.1 Hz, CHPh), 7.22-7.42 (9 H, m, Ph-H_5 + Ar-H_4); MS *m/z* 488.1255 (M + H) ($C_{25}H_{29}{}^{81}BrNO_4$ requires 488.1259), 486.1258 (M + H) ($C_{25}H_{29}{}^{79}BrNO_4$ requires 486.1279), 91 (Bn). Compound **41c** was condensed with guanidine, as for the synthesis of **42a**, to give **42c** (53%) as a highly hygroscopic buff solid: NMR δ_H 1.31 (3 H, s, Me), 1.34 (3 H, s, Me), 1.75 (1 H, m, C*H*CHO), 1.88 (1 H, m, C*H*CHO), 2.33 (1 H, ddd, *J* = 13.7, 10.5, 5.9 Hz, Pyr-CH), 2.48 (1 H, ddd, *J* = 13.7, 10.5, 5.9 Hz, Pyr-CH), 3.45-3.53 (2 H, m, CH_2OBn), 3.70 (1 H, dt, *J* = 7.8, 3.5 Hz, dioxolane 4-H), 3.77 (1 H, m, dioxolane 5-H), 4.51 (1 H, d, *J* = 12.1 Hz, CHPh), 4.57 (1 H, d, *J* = 12.1 Hz, CHPh), 4.76 (2 H, br, NH_2), 5.18 (2 H, br, NH_2), 7.09 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.28-7.38 (5 H, m, Ph-H_5), 7.54 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); MS *m/z* 515.1488 (M + H) ($C_{25}H_{30}{}^{81}BrN_4O_3$ requires 515.1480), 513.1500 (M + H) ($C_{25}H_{30}{}^{79}BrN_4O_4$ requires 513.1501), 487/485 (M – C_2H_5), 407/405 (M - C_7H_7O), 91 (Bn).

6.60. 6-((3*S*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-phenylpyrimidine-2,4-diamine (43a)

Compound **42a** was treated with aq. CF_3CO_2H, as for the synthesis of **26a**, to give **43a** (210 mg, 76%) as a pale buff solid: mp 101-102ºC; NMR (CD_3OD) δ_H 1.70 (1 H, q, *J* = 7.6 Hz, 2'-H_2), 2.36 (1 H, dt, *J* = 14.2, 7.6 Hz, 1'-H), 2.48 (1 H, dt, *J* = 14.2, 7.6 Hz, 1'-H), 3.42-3.55 (4 H, m, 3',4',5'-H_4), 4.48 (1 H, d, *J* = 11.7 Hz, CHPh), 4.52 (1 H, d, *J* = 11.7 Hz, CHPh), 7.22-

7.49 (10 H, m, 2 × Ph-H_5); MS *m/z* 395.2082 (M + H) ($C_{22}H_{27}N_4O_3$ requires 359.2083), 91 (Bn).

6.61. 6-((3*S*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (43b)

Compound **42b** was treated with aq. CF_3CO_2H, as for the synthesis of **26a**, to give **43b** (75%) as a pale yellow solid: mp 141-143°C; IR ν_{max} 3562, 3492, 3430, 3343, 1618 cm^{-1}; NMR (CD_3OD) δ_H 1.65-1.73 (2 H, m, 2'-H_2), 2.28 (1 H, ddd, *J* = 13.7, 9.4, 6.6 Hz, 1'-H), 2.42 (1 H, ddd, *J* = 13.7, 9.4, 6.6 Hz, 1'-H), 3.42-3.54 (4 H, m, 3',4',5'-H_4), 4.48 (1 H, d, *J* = 11.7 Hz, CHPh), 4.52 (1 H, d, *J* = 11.7 Hz, CHPh), 7.20 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.30-7.36 (5 H, m, Ph-H_5), 7.44 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); MS *m/z* 431.1680 (M + H) ($C_{22}H_{26}{}^{37}ClN_4O_3$ requires 431.1663), 429.1702 (M + H) ($C_{22}H_{26}{}^{35}ClN_4O_3$ requires 429.1693), 91 (Bn).

6.62. 6-((3*S*,4*S*)-5-Benzyloxy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine-2,4-diamine (43c)

Compound **42c** was treated with aq. CF_3CO_2H, as for the synthesis of **26a**, to give **43c** (95%) as a highly hygroscopic pale yellow solid: IR ν_{max} 3582, 3350, 1613 cm^{-1}; NMR (CD_3OD) δ_H 1.71-1.78 (2 H, m, 2'-H_2), 2.36 (1 H, dt, *J* = 14.6, 7.8 Hz, 1'-H), 2.46 (1H, dt, *J* = 14.6, 7.8 Hz, 1'-H), 3.43-3.61 (4 H, m, 3',4',5'-H_4), 4.51 (1 H, d, *J* = 11.9 Hz, CHPh), 4.56 (1 H, d, *J* = 11.9 Hz, CHPh), 7.18 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.34-7.36 (5 H, m, Ph-H_5), 7.63 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); NMR (CD_3OD) δ_C 29.26, 31.59, 70.80, 72.32, 71.11, 72.99, 107.30, 120.98, 122.10, 127.39, 127.58, 128.04, 130.83, 132.31, 138.05, 161.45, 161.80, 162.50; MS *m/z* 475.1184 (M + H) ($C_{22}H_{26}{}^{81}BrN_4O_3$ requires 475.1167), 473.1186 (M + H) ($C_{22}H_{26}{}^{79}BrN_4O_4$ requires 473.1188), 91 (Bn).

6.63. 1-Cyano-7-hydroxy-1-phenylheptan-2-one (45a) / 1-cyano-1-phenylhept-1-en-1,7-diol (46a)

Phenylacetonitrile and tetrahydrooxepin-2-one **44** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a**, to give **45a**/**46a** (21%) as a pale yellow solid: mp 98-99°C; IR ν_{max} 3402, 2205, 1718 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.35-1.42 (2 H, m, 5-H_2), 1.44-1.51 (2 H, m, 6-H_2), 1.65 (2 H, qn, *J* = 7.4 Hz, 4-H_2), 2.60 (2 H, t, *J* = 7.4 Hz, 3-H_2), 3.40 (2 H, t, *J* = 6.2 Hz, 7-H_2), 4.36 (1 H, br, OH), 7.20 (1 H, t, *J* = 7.6 Hz, Ph 4-H), 7.30 (2 H, t, *J* = 7.6 Hz, Ph 3,5-

H_2), 7.61 (2 H, d, *J* = 7.6 Hz, Ph 2,6-H_2); MS *m/z* 232.1329 (M + H) ($C_{14}H_{18}NO_2$ requires 232.1337), 214 (M - OH), 185 (M – C_2H_6O), 115 (M – $C_6H_{12}O_2$).

6.64. 1-(4-Chlorophenyl)-1-cyano-7-hydroxyheptan-2-one (45b) / 1-(4-chlorophenyl)-1-cyanohept-1-en-1,7-diol (46b)

4-Chlorophenylacetonitrile and tetrahydrooxepin-2-one **44** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a**, to give **45b**/**46b** (11%) as a white solid: mp 92-94°C; NMR δ_H 1.21-1.30 (2 H, m, 5-H_2), 1.50 (2 H, qn, *J* = 6.8 Hz, 6-H_2), 1.58 (2 H, qn, *J* = 7.4 Hz, 4-H_2), 2.58 (1 H, dt, *J* = 18.2, 7.4 Hz, 3-H), 2.66 (1 H, dt, *J* = 18.2, 7.4 Hz, 3-H), 3.60 (2 H, t, *J* = 6.8 Hz, 7-H_2), 4.65 (1 H, br, OH), 7.32 (2 H, d, *J* = 8.4 Hz, Ar 2,6-H_2), 7.41 (2 H, d, *J* = 8.4 Hz, Ar 3,5-H_2); MS *m/z* 268.0912 (M + H) ($C_{14}H_{17}{}^{37}ClNO_2$ requires 268.0918), 266.0942 (M + H) ($C_{14}H_{17}{}^{35}ClNO_2$ requires 266.0947), 250/248 (M – OH), 207/205 (M - C_3H_8O).

6.65. 1-(4-Bromophenyl)-1-cyano-7-hydroxyheptan-2-one (45c) / 1-(4-bromophenyl)-1-cyanohept-1-en-1,7-diol (46c)

4-Bromophenylacetonitrile and tetrahydrooxepin-2-one **44** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a** (chromatographic eluant EtOAc / hexane (3:1)), to give **45c**/**46c** (8%) as a pale yellow solid: mp 76-78°C; NMR δ_H 1.28 (2 H, qn, *J* = 7.3 Hz, 5-H_2), 1.50 (2 H, qn, *J* = 7.3 Hz, 6-H_2), 1.58 (2 H, qn, *J* = 7.3 Hz, 4-H_2), 2.62 (1 H, dt, *J* = 18.0, 7.3 Hz, 3-H), 2.65 (1 H, dt, *J* = 18.0, 7.3 Hz, 3-H), 3.60 (2 H, t, *J* = 6.4 Hz, 7-H_2), 4.64 (1 H, br, OH), 7.26 (2 H, d, *J* = 8.2 Hz, Ar 2,6-H_2), 7.41 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2); MS *m/z* 312.0430 (M + H) ($C_{14}H_{17}{}^{81}BrNO_2$ requires 312.0422), 310.0449 (M + H) ($C_{14}H_{17}{}^{79}BrNO_2$ requires 310.0442), 294/292 (M – OH).

6.66. 1-Cyano-1-(3,4-dichlorophenyl)-7-hydroxyheptan-2-one (45d) / 1-cyano-1-(3,4-chlorophenyl)hept-1-en-1,7-diol (46d)

3,4-Dichlorophenylacetonitrile and tetrahydrooxepin-2-one **44** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **45c**/**46c**, to give **45d**/**46d** (25%) as a pale yellow solid: mp 95-97°C; NMR δ_H 1.26 (2 H, qn, *J* = 7.3 Hz, 5-H_2), 1.53 (2 H, qn, *J* = 7.3 Hz, 6-H_2), 1.62 (2 H, qn, *J* = 7.3 Hz, 4-H_2), 2.66 (2 H, dt, *J* = 15.4, 7.3 Hz, 3-H_2), 3.60 (2 H, t, *J* = 6.4 Hz, 7-H_2), 4.69 (1 H, br, OH), 7.39 (1 H, d, *J* = 8.4 Hz, Ar 6-H), 7.51 (1 H, d, *J* = 8.4 Hz, Ar 5-H), 7.83 (1 H, s, Ar 2-H); MS *m/z* 304.0520 (M + H) ($C_{14}H_{16}{}^{37}Cl_2NO_2$ requires 304.0499),

302.0537 (M + H) ($C_{14}H_{16}{}^{37}Cl^{35}ClNO_2$ requires 302.0528), 300.0559 (M + H) ($C_{14}H_{16}{}^{35}Cl_2NO_2$ requires 300.0558), 286/284/282 (M – OH).

6.67. (4*R*,5*R*)-4-(2-Cyano-1-oxo-2-phenylethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49a) / (4*R*,5*R*)-4-(2-cyano-1-hydroxy-2-phenylethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50a)

Phenylacetonitrile and 2,3-*O*-isopropylidene–D-erythronolactone **48** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a**, to give **49a**/**50a** (27%) as a pale yellow oil: IR ν_{max} 3408, 2246, 1694 cm^{-1}; NMR δ_H 1.41 (3 H, s, Me), 1.49 (3 H, s, Me), 4.41 (1 H, dd, *J* = 11.0, 3.5 Hz, C*H*OH), 4.48 (1 H, d, *J* = 11.0 Hz, C*H*OH), 4.75 (1 H, d, *J* = 5.5 Hz, 4-H), 4.88 (1 H, m, 5-H), 7.47 (2 H, t, *J* = 7.4 Hz, Ph 3,5-H_2), 7.61 (1 H, t, *J* = 7.4 Hz, Ph 4-H), 8.10 (2 H, d, *J* = 8.6 Hz, Ph 2,6-H_2); MS *m/z* 276.1225 (M + H) ($C_{15}H_{18}NO_4$ requires 276.1235), 258 (M – OH).

6.68. (4*R*,5*R*)-4-(2-(4-Chlorophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49b) / (4*R*,5*R*)-4-(2-(4-chlorophenyl)-2-cyano-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50b)

4-Chlorophenylacetonitrile and 2,3-*O*-isopropylidene–D-erythronolactone **48** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a**, to give **49b**/**50b** (21%) as a pale yellow oil: NMR δ_H 1.30 (3 H, s, Me), 1.59 (3 H, s, Me), 4.05 (1 H, dd, *J* = 10.1, 3.1 Hz, C*H*OH), 4.09 (1 H, d, *J* = 10.1 Hz, C*H*OH), 4.86 (1 H, m, 5-H), 4.73 (1 H, d, *J* = 5.8 Hz, 4-H), 7.27 (2 H, d, *J* = 8.4 Hz, Ar 2,6-H_2), 7.48 (2 H, d, *J* = 8.4 Hz, Ar 3,5-H_2); MS *m/z* 312.0849 (M + H) ($C_{15}H_{17}{}^{37}ClNO_4$ requires 312.0816), 310.0855 (M + H) ($C_{15}H_{16}{}^{35}ClNO_4$ requires 310.0846), 294/292 (M – OH).

6.69. (4*R*,5*R*)-4-(2-(4-Bromophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49c) / (4*R*,5*R*)-4-(2-(4-bromophenyl)-2-cyano-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50c)

4-Bromophenylacetonitrile and 2,3-*O*-isopropylidene–D-erythronolactone **48** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a** (chromatographic eluant EtOAc / hexane (3:1)), to give **49c**/**50c** (38%) as a pale yellow oil: IR ν_{max} 3422, 2208, 1777 cm^{-1}; NMR δ_H 1.39 (3 H, s, Me), 1.47 (3 H, s, Me), 4.40 (1 H, dd, *J* = 10.9, 3.7 Hz, C*H*OH), 4.45 (1 H, d, *J* = 10.9 Hz, *CH*OH), 4.74 (1 H, d, *J* = 5.5 Hz, 4-H), 4.87 (1 H, m, 5-H), 5.57 (1 H, s, CHCN),

7.27 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.51 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); MS *m/z* 356.0326 (M + H) ($C_{15}H_{17}{}^{81}BrNO_4$ requires 356.0320), 354.0327 (M + H) ($C_{15}H_{17}{}^{79}BrNO_4$ requires 354.0340), 338/336 (M –OH).

6.70. (4*R*,5*R*)-4-(2-Cyano-2-(3,4-dichlorophenyl)-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49d) / (4*R*,5*R*)-4-(2-cyano-2-(3,4-dichlorophenyl)-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50d)

3,4-Dichlorophenylacetonitrile and 2,3-*O*-isopropylidene–D-erythronolactone **48** were treated with $LiN(SiMe_3)_2$, as for the synthesis of **22a**/**23a** (chromatographic eluant EtOAc / hexane (1:1)), to give **49d**/**50d** (22%) as a pale yellow oil: IR ν_{max} 3404, 2250, 1782 cm^{-1}; NMR δ_H 1.30 (3 H, s, Me), 1.38 (3 H, s, Me), 3.93 (1 H, dd, *J* = 10.3, 3.7 Hz, C*H*OH), 4.00 (1 H, d, *J* = 10.3 Hz, C*H*OH), 4.70 (1 H, d, *J* = 5.9 Hz, 4-H), 4.91 (1 H, dd, *J* = 5.9, 3.7 Hz, 5-H), 7.33 (1 H, dd, *J* = 8.2, 2.0 Hz, Ar 6-H), 7.36 (1 H, d, *J* = 8.2 Hz, Ar 5-H), 7.58 (1 H, d, *J* = 2.0 Hz, Ar 2-H); MS *m/z* 348.0411 (M + H) ($C_{15}H_{16}{}^{37}Cl_2NO_4$ requires 348.0397), 346.0901 (M + H) ($C_{15}H_{16}{}^{37}Cl^{35}ClNO_4$ requires 346.0906), 344.0448 (M + H) ($C_{15}H_{16}{}^{35}Cl_2NO_4$ requires 344.0456), 330/328/326 (M – OH).

6.71. (4*R*,5*R*)-4-(2-Cyano-1-methoxy-2-phenylethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (51a) and 6-((4*S*,5*R*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)-5-phenylpyrimidine-2,4-diamine (52a)

Compound **49a**/**50a** was treated with CH_2N_2, as for the synthesis of **24a**, to give **51a** (85%) as a pale yellow oil: IR ν_{max} 3492, 2209 cm^{-1}; NMR δ_H 1.44 (3 H, s, 2-Me), 1.58 (3 H, s, 2-Me), 3.53 (3 H, s, OMe), 4.39 (1 H, dd, *J* = 11.0, 3.7 Hz, C*H*OH), 4.45 (1 H, d, *J* = 11.0 Hz, C*H*OH), 4.60 (1 H, m, 5-H), 5.37 (1 H, d, *J* = 7.4 Hz, 4-H), 7.30-7.41 (5 H, m, Ph-H_5); MS *m/z* 290.1388 (M + H) ($C_{16}H_{20}NO_4$ requires 290.1392), 274 (M – Me), 258 (M - OMe). Compound **51a** was treated with guanidine, as for the synthesis of **25a** (chromatographic eluant CH_2Cl_2 / MeOH (4:1)) to give **52a** (48%) as a pale yellow solid: mp 214-216ºC; IR ν_{max} 3492, 3465, 3422, 3318, 3178, 1624 cm^{-1}; $[\alpha]^{20}_D$ = +3.3° (c 4, $CHCl_3$); NMR δ_H 1.21 (3 H, s, Me), 1.62 (3 H, s, Me), 3.48 (1 H, dd, *J* = 12.7, 2.1 Hz, C*H*OH), 3.57 (1 H, dd, *J* = 12.7, 3.3 Hz, C*H*OH), 3.97 (1 H, m, dioxolane 5-H), 4.79 (1 H, d, *J* = 6.6 Hz, dioxolane 4-H), 4.90 (2 H, br, NH_2), 5.16 (2 H, br, NH_2), 7.10 (1 H, d, *J* = 7.4 Hz, Ph 2-H), 7.31 (1 H, d, *J* = 7.4 Hz, Ph 6-H), 7.41 (1 H, t, *J* = 7.4 Hz, Ph 4-H), 7.47 (2 H, t, *J* = 7.4 Hz, Ph 3,5-H_2); MS *m/z* 317.1622 (M + H) ($C_{16}H_{20}N_4O_3$ requires 317.1613).

6.72. (4*R*,5*R*)-4-(2(4-Chlorophenyl)-2-cyano-1-methoxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (51b) and 5-(4-chlorophenyl)-6-((4*S*,5*R*)-2,2-dimethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52b)

Compound **49b**/**50b** was treated with CH_2N_2, as for the synthesis of **24a**, to give **51b** (69%) as a pale yellow oil: MS *m/z* 326.0989 (M + H) ($C_{16}H_{19}{}^{37}ClNO_4$ requires 326.0973), 324.1014 (M + H) ($C_{16}H_{19}{}^{35}ClNO_4$ requires 324.1002), 307/305 (M - H_2O). Compound **51b** was treated with guanidine, as for the synthesis of **52a**, to give **52b** (50%) as a pale buff solid: mp 172-174ºC; IR ν_{max} 3497, 3459, 3433, 3396, 3217, 1613 cm^{-1}; NMR δ_H 1.24 (3 H, s, Me), 1.63 (3 H, s, Me), 1.66 (1 H, br, OH), 3.47 (1 H, dd, *J* = 12.8, 2.3 Hz, C*H*OH), 3.58 (1 H, dd, *J* = 12.8, 3.3 Hz, C*H*OH), 3.98 (1 H, m, dioxolane 5-H), 4.66 (2 H, br, NH_2), 4.77 (1 H, d, *J* = 6.2 Hz, dioxolane 4-H), 4.93 (2 H, br, NH_2), 7.05 (1 H, dd, *J* = 8.8, 2.0 Hz, Ar 2-H), 7.27 (1 H, dd, *J* = 9.4, 2.0 Hz, Ar 6-H), 7.43 (1 H, dd, *J* = 8.8, 2.0 Hz, Ar 3-H), 7.47 (1 H, dd, *J* = 9.4, 2.0 Hz, Ar 5-H); MS *m/z* 353.1218 (M + H) ($C_{16}H_{20}{}^{37}ClN_4O_3$ requires 353.1194), 351.1236 (M + H) ($C_{16}H_{20}{}^{35}ClN_4O_3$ requires 351.1223), 295/293 (M – C_3H_5O).

6.73. (4*R*,5*R*)-4-(2-(4-Bromophenyl)-2-cyano-1-methoxyethenyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (51c) and 5-(4-bromophenyl)-6-((4*S*,5*R*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52c)

Compound **49c**/**50c** was treated with CH_2N_2, as for the synthesis of **24a**, to give **51c** (91%) as a pale yellow oil: NMR δ_H 1.44 (3 H, s, 2-Me), 1.48 (3 H, s, 2-Me), 3.57 (3 H, s, OMe), 4.40 (1 H, dd, *J* = 10.9, 3.5 Hz, C*H*OH), 4.46 (1 H, d, *J* = 10.9 Hz, C*H*OH), 4.61 (1 H, m, 5-H), 5.34 (1 H, d, *J* = 7.0 Hz, 4-H), 7.36 (2 H, d, *J* = 8.6 Hz, Ar 2,6-H_2), 7.50 (2 H, d, *J* = 8.6 Hz, Ar 3,5-H_2); MS *m/z* 370.0481 (M + H) ($C_{16}H_{19}{}^{81}BrNO_4$ requires 370.0476), 368.0501 (M + H) ($C_{16}H_{19}{}^{79}BrNO_4$ requires 368.0497). Compound **51c** was treated with guanidine, as for the synthesis of **52a**, to give **52c** (29%) as a pale yellow solid: mp 181-183ºC; NMR ($(CD_3)_2SO$) δ_H 1.14 (3 H, s, Me), 1.48 (3 H, s, Me), 3.45 (2 H, d, *J* = 3.5 Hz, CH_2O), 4.03 (1 H, dt, *J* = 6.4, 3.5 Hz, dioxolane 5-H), 4.73 (1 H, d, *J* = 6.4 Hz, dioxolane 4-H), 5.70 (2 H, br, NH_2), 5.85 (2 H, br, NH_2), 7.20 (1 H, dd, *J* = 8.1, 2.0 Hz, Ar 2-H), 7.25 (1 H, dd, *J* = 7.7, 2.0 Hz, Ar 6-H), 7.61 (1 H, dd, *J* = 7.7, 2.0 Hz, Ar 5-H), 7.63 (1 H, dd, *J* = 8.1, 2.0 Hz, Ar 3-H); NMR ($(CD_3)_2SO$) δ_C 25.44, 26.37, 62.49, 76.46, 79.81, 108.35, 108.72, 121.70, 132.30, 132.35, 132.59, 133.38, 134.30, 159.21, 162.29, 163.21; MS *m/z* 397.0694 (M + H) ($C_{16}H_{20}{}^{81}BrN_4O_3$ requires 397.0698), 395.0712 (M + H) ($C_{16}H_{20}{}^{79}BrN_4O_3$ requires 395.0718).

6.74. (4*R*,5*R*)-2-Cyano-4-(2-(3,4-dichlorophenyl)-1-methoxyethenyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (51d) and 5-(3,4-dichlorophenyl)-6-((4*S*,5*R*)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52d)

Compound **49d**/**50d** was treated with CH_2N_2, as for the synthesis of **24a**, to give **51d** (78%) as a pale yellow oil: IR ν_{max} 3534, 2247, 1595 cm^{-1}; NMR δ_H 1.44 (3 H, s, 2-Me), 1.47 (3 H, s, 2-Me), 3.72 (3 H, s, OMe), 4.54 (1 H, dd, *J* = 10.7, 4.1 Hz, C*H*OH), 4.73 (1 H, d, *J* = 10.7 Hz, C*H*OH), 4.96 (1 H, m, 5-H), 5.52 (1 H, d, *J* = 5.9 Hz, 4-H), 7.15 (1 H, d, *J* = 8.5 Hz, Ar 5-H), 7.37 (1 H, dd, *J* = 8.5, 1.4 Hz, Ar 6-H), 7.40 (1 H, d, *J* = 1.4 Hz, Ar 2-H); MS *m/z* 360.0378 (M - H) ($C_{16}H_{16}{}^{37}Cl_2NO_4$ requires 360.0397), 358.0433 (M - H) ($C_{16}H_{16}{}^{37}Cl^{35}ClNO_4$ requires 358.0426), 356.0452 (M - H) ($C_{16}H_{16}{}^{35}Cl_2NO_4$ requires 356.0456), 346/344/342 (M – Me). Compound **51d** was treated with guanidine, as for the synthesis of **52a**, to give **52d** (47%) as a pale yellow solid: mp 181-183ºC; NMR (CD_3CN) δ_H 1.20 (3 H, s, Me), 1.49 (3 H, s, Me), 3.40-3.42 (2 H, m, CH_2OH), 4.03 (1 H, m, 5-H), 4.72 (1 H, d, *J* = 7.0 Hz, 4-H), 5.21 (2 H, br, NH_2), 5.31 (2 H, br, NH_2), 7.11 (0.5 H, dd, *J* = 8.2, 2.0 Hz, Ph 6-H), 7.21 (0.5 H, dd, *J* = 8.2, 2.0 Hz, Ph 6-H), 7.38 (0.5 H, d, *J* = 2.0 Hz, Ph 2-H), 7.48 (0.5 H, d, *J* = 2.0 Hz, Ph 2-H), 7.60 (0.5 H, d, *J* = 8.2 Hz, Ph 5-H), 7.62 (0.5 H, d, *J* = 8.2 Hz, Ph 5-H); NMR ($(CD_3)_2CO$) δ_C 24.90 (Me), 25.88 (Me), 61.93 (CH_2OH), 76.13 (CH), 79.16 (CH), 107.43 (*C*Me_2), 108.75 (Pyr 5-C), 130.36 (Ph C), 131.07 (Ph CH), 131.18 (Ph C), 131.51 (Ph CH), 132.89 (Ph CH), 135.14 (Ph C), 159.37 (Pyr 2-C), 161.93 (Pyr 4-C), 162.93 (Pyr 6-C); MS *m/z* 389.0778 (M + H) ($C_{16}H_{19}{}^{37}Cl_2N_4O_3$ requires 389.0775), 387.0810 (M + H) ($C_{16}H_{19}{}^{37}Cl^{35}ClN_4O_3$ requires 387.0833), 385.0833 (M + H) ($C_{16}H_{19}{}^{35}Cl_2N_4O_3$ requires 385.0834), 331/329/327 (M – C_3H_5O).

6.75. 1-Cyano-1,4-diphenylbutan-2-one (54) / 1-cyano-1,4-diphenylbut-1-en-2-ol (55)

Phenylacetonitrile was condensed with ethyl 3-phenylpropanoate **53**, as for the synthesis of **22a**/**23a**, to give **54**/**55** (34%) as a pale buff solid: mp 53-54ºC (lit.[54] mp 76-78°C) ; IR ν_{max} 2200 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 2.88 (2 H, t, *J* = 6.4 Hz, CH_2), 2.94 (2 H, t, *J* = 6.4 Hz, CH_2), 7.20-7.61 (10 H, m, 2 × Ph-H_5), 11.70 (1 H, br s, OH) MS *m/z* 250.1240 (M + H) ($C_{17}H_{16}NO$ requires 250.1231), 222 (M – HCN), 91 (Bn).

6.76. 1-Cyano-1-phenylpropan-2-one (58) / 1-cyano-1-phenylprop-1-en-2-ol (59)

Phenylacetonitrile was condensed with ethyl acetate **58**, as for the synthesis of **22a**/**23a** except

that chromatography was omitted and the product was recrystallised (aq. EtOH), to give **58**/**59** (31%) as a pale buff solid: mp 87-88ºC (lit.[55] mp 87-89ºC); NMR δ_H 2.25 (3 H, s, Me), 4.66 (1 H, s, CHCN), 7.38-7.47 (5 H, m, Ph-H_5); MS *m/z* 160.0740 (M + H) ($C_{10}H_{10}NO$ requires 160.0762), 144 (M - CH_3), 118 (M – C_2H_3N).

6.77. 1-(4-Chlorophenyl)-1-cyanobutan-2-one (68) / 1-(4-chlorophenyl)-1-cyano-but-1-en-2-ol (64)

4-Chlorophenylacetonitrile was condensed with ethyl propanoate **62**, as for the synthesis of **22a**/**23a**, to give **62**/**63** (31%) as a pale yellow solid: mp 50-51ºC (lit.[16] mp 50-52ºC); NMR ($(CD_3)_2SO$) δ_H 1.24 (3 H, t, *J* = 7.4 Hz, Me), 2.62 (2 H, q, *J* = 7.4 Hz, CH_2), 7.42 (2 H, d, *J* = 8.8 Hz, 2,6-H_2), 7.66 (2 H, d, *J* = 8.8 Hz, 3,5-H_2).

6.78. Biological assay

The radial spoke assay was performed essentially as described by Gerum *et al*.[38] and Sibley *et al*.[56] The three yeasts were grown in media comprising 10% yeast extract, 10% peptone and 10% dextrose. Sulfanilamide (1.0 mM, 100 μL), an inhibitor of dihydropteroate synthase,[57] was spread onto fresh agar plates and allowed to absorb into the medium overnight. Three template plates were streaked with the yeast cultures in two orthogonal lines and incubated at 30°C for 3 d. These plates were used to generate replica test plates. Test compounds **7a-d**, **8a-c**, **9a-d**, **10a-d** and control compounds **3**, **6**, **11**, **12** were made up as 10 mM solutions in DMSO; a spot (10 μL) of each of these solutions was placed at the centre of each test plate. The assay plates were then incubated for 3 days at 30ºC before the inhibition zone was measured. Each compound/yeast combination was assayed in triplicate.

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References

1. Srivastava, R.; Kumar, D.; Subramaniam, P.; Srivastava, B. S. *Biochem. Biophys. Res. Comm.* **1997**, *235*, 602.

2. Janin, Y. L. *Bioorg. Med. Chem.* **2007**, *15*, 2479.

3. Crofton, J. *J. Pharm. Pharmacol*. **1997**, *49*, 3.

4. Takayama, K.; Kilburn, J. O. *Antimicrob. Agents Chemother*. **1989**, *33*, 1493.

5. Wolucka, B. A.; McNeil, M. R.; de Hoffmann, E.; Chojnacki, T.; Brennan, P. J. *J. Biol. Chem*. **1994**, *269*, 23328.

6. DeCock, K. M.; Chaisson, R. E. *Int. J. Tubercul. Lung Dis*. **1999**, *3*, 457.

7. Zhang, Y.; Amzel, L. M. *Current Drug Targets* **2002**, *3*, 131.

8. Kompis, I. M.; Islam, K.; Then, R. L. *Chem. Rev.* **2005**, *105*, 593.

9. Blaney, J. M.; Hansch, C.; Silipo, C.; Vittoria, A. *Chem. Rev.* **1984**, *84*, 333.

10. Tendler, S. J. B.; Threadgill, M. D.; Tisdale, M. J. *Cancer Lett.* **1987**, *36*, 65.

11. Zanetti, K. A.; Stover, P. J. *J. Biol. Chem.* **2003**, *278*, 10142.

12. McGuire, J. J. *Current Pharm. Des.* **2003**, *9*, 2593.

13. Then, R. L. *J. Chemother.* **2004**, *16*, 3.

14. Robson, C.; Meek, M. A.; Grunwaldt, J.-D.; Lambert, P. A.; Queener, S. A.; Schmidt, D.; Griffin, R. J. *J. Med. Chem.* **1997**, *40*, 3040.

15. White, E. L.; Ross, L. R.; Cunningham, A.; Escuyer, V. *FEMS Microbiol. Lett.* **2004**, *232*, 101.

16. Russell, P. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1951**, *73*, 3763.

17. Sardarian, A.; Douglas, K. T.; Read, M.; Sims, P. F. G.; Hyde, J. E.; Chitnumsub, P.; Sirawaraporn, R.; Sirawaraporn, W. *Org. Biomol. Chem.* **2003**, *1*, 960.

18. Denny, B. J.; Ringan, N. S.; Schwalbe, C. H.; Lambert, P. A.; Meek, M. A.; Griffin, R. J.; Stevens, M. F. G. *J. Med. Chem.* **1992**, *35*, 2315.

19. Tarnchompoo, B.; Sirichaiwat, C.; Phupong, W.; Intaraudom, C.; Sirawaraporn, W.; Kamchonwongpaisan, S.; Vanichtanankul, J.; Thebtaranonth, Y.; Yuthavong, Y. *J. Med. Chem.* **2002**, *45*, 1244.

20. Haller, L.; Sossouhounto, R.; Coulibaly, I. M.; Dosso, M.; Kone, M.; Adom, H.; Meyer, U. A.; Betschart, B.; Wenk, M.; Haefeli, W. E.; Lobognon, L. R.; Porquet, M.; Kabore, G.; Sorenson, F.; Reber-Liske, R.; Sturchler, D. *Chemotherapy* **1999**, *45*, 452.

21. Argyrou, A.; Vetting, M. W.; Aladegbami, B.; Blanchard, J. S. *Nature Struct. Mol. Biol.* **2006**, *13*, 408.

22. Lynch, G.; Kemeny, N.; Currie, V. *Cancer Treat. Rep.* **1981**, *65*, 127.

23. Zawilska, J.; Nowak, J. Z. *Polish J. Pharmacol. Pharm.* **1985**, *37*, 821.

24. Horton, J. R.; Sawada, K.; Nishibori, M.; Cheng, X. *J. Mol. Biol.* **2005**, *353*, 334.

25. Laszlo, J.; Fyfe, M. J.; Sedwick, D.; Lee, L.; Brown, O. *Cancer Treat. Rep.* **1978**, *62*, 341.

26. Malik, N. S.; Matlin, S. A.; Fried, J.; Pakyz, R. E.; Consentino, M. J. *J. Androl.* **1995**, *16*, 169.

26. Mdluli, K.; Spigelman, M. *Curr. Opinion Pharmacol.* **2006**, *6*, 459.

27. Li, R.; Sirawaraporn, R.; Chitnumsub, P.; Sirawaraporn, W.; Wooden, J.; Athappilly, F.; Turley, S.; Hol, W. G. J. *J. Mol. Biol.* **2000**, 295, 307.

28. Arcus, V. L.; Lott, J. S.; Johnston, J. M.; Baker, E. N. *Drug Discovery Today* **2006**, *11*, 28.

29. da Cunha, E. F. F.; Ramalho, T. C.; de Alencastro, R. B.; Maia, E. R. *J. Biomol. Struct. Dynamics* **2004**, *22*, 119.

30. Suling, W. J.; Seitz, L. E.; Pathak, V.; Westbrook, L.; Barrow, E. W.; Zywno-van-Ginkel, S.; Reynolds, R. C.; Piper, J. R.; Barrow, W. W. *Antimicrob. Agents Chemother.* **2000**, *44*, 2784.

31. Cody, V.; Galitsky, N.; Luft, J. R.; Pangborn, W.; Rosowsky, A.; Blakley, R. L. *Biochemistry* **1997**, *36*, 13897.

32. Davies, J. F. D.; Delcamp, T. J.; Prendergast, N. J.; Ashford, V. A.; Frisheim, J. H.; Kraut, J. *Biochemistry* **1990**, *29*, 9467.

33. Shah, L. M.; Meyer, S. C.; Cynamon, M. H. *Antimicrob. Agents Chemother.* **1996**, *40*, 2426.

34. Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469.

35. Park, M. H.; Takeda, R.; Nakanishi, K. *Tetrahedron Lett*. **1987**, *28*, 3823.

36. Threadgill, M. D.; Griffin, R. J.; Stevens, M. F. G.; Wong, S. K. *J. Chem. Soc., Perkin Trans. 1*, **1987**, 2229.

37. Gerum, A. B.; Ulmer, J. E.; Jacobus, D. P.; Jensen, N. P.; Sherman, D. R.; Sibley, C. H. *Antimicrob. Agents Chemother*. **2002**, *46*, 3362.

38. Cren, S.; Gurcha, S. S.; Blake, A. J.; Besra, G. S.; Thomas, N. R. *Org. Biomol. Chem.* **2004**, *2*, 2418.

39. Malik, M.; Lu, T.; Zhao, X.; Singh, A.; Hattan, C. M.; Domaglia, J.; Kerns, R.; Drlica, K. *Antimicrob. Agents Chemother*. **2005**, *49*, 2008.

40. Suling, W. J.; Seitz, L. E.; Reynolds, R. C.; Barrow, W. W. *Antimicrob. Agents Chemother*. **2005**, *49*, 4801.

41. Quan, S.; Venter, H.; Dabbs, E. R. *Antimicrob. Agents Chemother*. **1997**, *41*, 2456.

42. Mdluli, K.; Sherman, D. R.; Hickey, M. J.; Kreiswirth, B. N.; Morris, S.; Stover, C. K. Barry, C. E. *J. Infect. Dis*. **1996**, *174*, 1085.

43. Hawser, S.; Lociuro, S.; Islam, K. *Biochem. Pharmacol.* **2006**, *71*, 941.

44. Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *J. Org. Chem*. **1990**, *55*, 4683.

45. Batty, D.; Crich, D. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3193.

46. Carmack, M.; Kelley, C. J. *J. Org. Chem*. **1968**, *33*, 2171.

47. Hungerbuhler, E.; Seebach, D. *Helv. Chim. Acta* **1981**, *64*, 687.

48. Kataky, R.; Nicholson, P. E.; Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1990**, 321.

49. Morimoto, T.; Chiba, M.; Achiwa, K. *Chem. Pharm. Bull*. **1993**, *41*, 1149.

50. Achmatowicz, B.; Wicha, J. *Bull. Pol. Acad. Sci. Chem*. **1988**, *36*, 267.

51. Saksena, A. K.; Lovey, R. G.; Girijavallabhan, V. M.; Ganguly, A. K.; McPhail, A. T. *J. Org. Chem*. **1986**, *51*, 5024.

52. Clough, S.; Raggatt, M. E.; Simpson, T. J.; Willis, C. L.; Whiting, A.; Wrigley, S. K. *J. Chem. Soc. Perkin Trans. 1* **2000**, 2475.

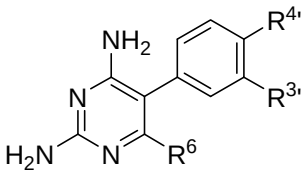
53. Haworth, R. D.; Mavin, C. R.; Sheldrick, G. *J. Chem. Soc*. **1934**, 1423.

54. Falcon, E. A.; Russell, P.B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1951**, *73*, 3753.

55. Sibley, C. H.; Brophy, V. H.; Cheesman, S.; Hamilton, K. L.; Hankins, E. G.; Wooden, J. M.; Kilbey, B. *Methods* **1997**, *13*, 190.

56. Gonzalez, A. H.; Berlin, O. G.; Bruckner, D. A. *J. Antimicrob. Chemother*. **1989**, *24*, 19.

Table 1. Diameters of zones of inhibition of growth of *S. cerevisiae* carrying the DHFR gene from *M. tuberculosis*, *S. cerevisiae* carrying the human DHFR gene and wild-type *S. cerevisiae* by test pyrimidine-2,4-diamines **7-10** and by control pyrimidine-2,4-diamines **11**, **12**, **3** (pyrimethamine) and **6** (trimethoprim).



Compound	$R^{3'}$	$R^{4'}$	R^6	Diameter of zone of inhibition (mm) *S. cerevisiae* (TB-DHFR)[a,b]	Diameter of zone of inhibition (mm) *S. cerevisiae* (human-DHFR)[a,c]	Diameter of zone of inhibition (mm) *S. cerevisiae* (yeast-DHFR)[a,d]
7a	H	H	(3*R*,4*S*)-3,4,5-trihydroxypentyl	11	6	6
7b	H	Cl	(3*R*,4*S*)-3,4,5-trihydroxypentyl	9	7	7
7c	H	Br	(3*R*,4*S*)-3,4,5-trihydroxypentyl	7	6	8
7d	Cl	Cl	(3*R*,4*S*)-3,4,5-trihydroxypentyl	8	8	8
8a	H	H	(3*S*,4*S*)-3,4,5-trihydroxypentyl	9	8	8
8b	H	Cl	(3*S*,4*S*)-3,4,5-trihydroxypentyl	7	7	6
8c	H	Br	(3*S*,4*S*)-3,4,5-trihydroxypentyl	8	5	6
9a	H	H	5-hydroxypentyl	7	7	9
9b	H	Cl	5-hydroxypentyl	8	8	8
9c	H	Br	5-hydroxypentyl	8	8	9

9d	Cl	Cl	5-hydroxypentyl	9	9	9
10a	H	H	(1*S*,2*R*)-1,2,3-trihydroxypropyl	5	5	5
10b	H	Cl	(1*S*,2*R*)-1,2,3-trihydroxypropyl	5	5	5
10c	H	Br	(1*S*,2*R*)-1,2,3-trihydroxypropyl	5	5	5
10d	Cl	Cl	(1*S*,2*R*)-1,2,3-trihydroxypropyl	8	8	7
20	H	H	2-phenylethyl	5	5	5
12	H	H	Me	7	8	8
3 (pyrimethamine)	Cl	H	ethyl	5	6	5
6 (trimethoprim)				5	6	6
DMSO negative control				5	6	5

[a] Diameters of the zone of inhibition were measured for each of the orthogonal streaks on each of at least three test plates for each determination; data are expressed ± 1 mm.

[b] TB5 yeast engineered to contain DHFR from *M. tuberculosis* only.

[c] TB5 yeast engineered to contain human DHFR only.

[c] TB5 yeast engineered to contain yeast DHFR only.